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Production of Mutagenic Compounds as a Result of Urban Photochemistry

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THE PRODUCTION OF MUTAGENIC COMPOUNDS AS A RESULT OF URBAN PHOTOCHEMISTRY

by

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16. ABSTRACT

A series of atmospheric simulation experiments was conducted to examine the role of urban photochemical processes on the formation and removal of potentially hazardous air pollutants. The experiments were conducted in a 22.7-m^3 Teflon smog chamber which was coupled to bioassay exposure chambers. The mutagenic activities of the tested mixtures of organic chemicals and nitrogen oxides were measured before and after irradiation by direct exposure of Salmonella typhimurium (strains TA 98 and TA 100) to the smog chamber contents. The mutagenic responses of the starting materials and the transformed products were quantified by using a modified Ames test. The chemicals examined included ubiquitous urban pollutants (e.g., propylene, toluene, and acetaldehyde), a potentially hazardous chlorinated solvent (allyl chloride), and complex mixtures (wood smoke and auto exhaust) from common urban pollutant sources. In all cases, the irradiated products were more mutagenic than the original chemicals. For the transformed complex mixtures, the bulk of the mutagenicity was found to be associated with the gas-phase products rather than with the aerosol-bound chemicals. The amount of mutagenicity was also observed to increase with increasing photochemical oxidation. The common photochemical pollutant, peroxyacetyl nitrate (PAN), was found to contribute significantly to the overall vapor-phase mutagenicity in all of the chemical systems in which it was formed.

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ABSTRACT

A series of hydrocarbon/NO_x irradiations were conducted in a 22.7-m^3 Teflon smog chamber in which the mutagenic activities (measured using the Ames test bacteria *Salmonella typhimurium* Strains TA100 and TA98) of the mixtures were measured before and after the irradiation. Simple hydrocarbons including propylene, acetaldehyde, toluene, and allyl chloride, as well as two complex hydrocarbon mixtures, wood smoke and automobile exhaust, were examined in detail. For these systems the irradiated mixtures were much more mutagenic than the reactant hydrocarbons. For the complex mixtures the distribution of mutagenic activity between the gas and particulate phase was measured, and, for all cases, a majority of the mutagenic activity of the product mixture was found in the gas phase. For each system studied, the observed mutagenic activities were interpreted in terms of the product species that may have contributed to the observed response. In most cases (except allyl chloride), peroxyacetyl nitrate was believed to contribute significantly.

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LIST OF ABBREVIATIONS

PAH	polycylic aromatic hydrocarbon
PAN	peroxyacetyl nitrate
HAP	hazardous air pollutant
PPN	peroxypropionyl nitrate
PBN	peroxybutyryl nitrate
PBzN	peroxybenzoyl nitrate
CPAN	chloroperoxyacetyl nitrate
PGDN	propylene glycol dinitrate
2-HPN	2-hydroxypropyl nitrate
2-NPA	2-nitratopropyl alcohol
NPPN	nitroxyperoxylpropyl nitrate
EAA	electrical aerosol analyzer
CNC	condensation nuclei counter

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SECTION 1

INTRODUCTION

Over the last several years, there has been an increased level of concern that exposure to polluted urban atmospheres may pose a "significant, although not yet precisely defined" threat to human health (1). Risk assessments (2,3) have tended to focus on the threat of developing cancer from exposure to vapor-phase air toxics that are emitted directly from urban sources. Efforts at chemical and biological characterization of ambient samples, however, have primarily examined the aerosol-bound species (4-6). This analytical emphasis on particulate pollutants was probably due to the greater ease of sampling, chemically analyzing, and conducting bioassays of the easily concentrated aerosol-bound organic compounds. Limited data on the biological impact of vapor-phase pollutants (7) in actual ambient air masses do indicate a significantly increased level of mutagenic activity in heavily polluted and industrialized areas. Clearly, any comprehensive assessment of the potential health impact resulting from exposure to air toxics must consider both the gaseous and particulate-bound chemicals.

In addition, it has become increasingly apparent that exposure and risk assessments must also consider the impact of atmospheric reactions on both hazardous and nonhazardous air pollutants. Haemisegger *et al.* (2) found a significant risk from exposure to formaldehyde, an air-toxic compound that is both directly emitted from combustion sources and is also formed from the photochemical oxidation of the many hydrocarbons present in urban air. The α,β-dicarbonyl compound, glyoxal, is a photooxidation product of a wide variety of important atmospheric aromatic hydrocarbons (e.g., toluene) and has also been shown to be mutagenic (8). Researchers have also found that a variety of oxygenated and nitrogenated species caused mutagenicity in the Ames test (9). These classes of compounds are continuously created and removed during the normal photooxidation of hydrocarbons. The atmospheric transformation of many ubiquitous urban air pollutants may, therefore, contribute significantly to the presence of mutagens in the ambient air, even though they, themselves, may be harmless.

Recent studies have shown that human exposure to wood stove and fireplace emissions may be a cause for public health concern. In a study of the Denver "brown cloud," Wolff *et al.* (10) determined that a significant fraction of the organic fine particulate matter (<2.5 μm) from Denver aerosol could be attributed to wood smoke emissions. In addition, particulate extracts from wood smoke have been tested for mutagenic activity by using the Ames test (11-13). These studies have

generally shown low direct-acting mutagenic activity with *Salmonella typhimurium* Strain TA98, although Dasch (11) reported substantial increases with the addition of S9 metabolic activation. In addition to the possible presence of mutagens in the emissions themselves, evidence has appeared recently in the literature indicating that reactions of species such as O_3 and N_2O_5 on the surface of atmospheric particulate matter can lead to increases in the mutagenic activity of adsorbed species such as PAHs (14-19). In addition, Bell and Kamens (20) and Kamens *et al.* (21) demonstrated that PAHs adsorbed on wood smoke particulate matter can photodegrade at significant rates. Clearly, atmospheric reactions can have a significant impact on the mutagenic activity of atmospheric particulate matter.

Although there is some evidence (7) of significant concentrations of gas-phase mutagens in ambient air, there has been a relatively small effort aimed at identifying mechanisms for the production of gas-phase mutagens through atmospheric photochemistry compared to efforts in the area of particulate-phase mutagenesis. To test for the mutagenic activity of gas-phase species by using the standard plate incorporation test (22), it is necessary to concentrate the volatile air pollutants from the air sample into a suitable solvent for testing. Some attempts to measure gas-phase ambient mutagenicities have employed a solid sorbent, such as XAD, to first collect the species and then to extract them into a solvent. However, these processes pose a number of potential problems, especially loss of volatile species during the extraction and workup procedures.

In this report we present a review of the results of experiments we have conducted by using an alternative technique for measuring the mutagenic activity of gas-phase species generated by photochemical reactions. For this technique, the Ames assay test plates are dosed continuously by an experimental air mass flowing over the uncovered plates, thereby enabling the soluble species to deposit continuously during the exposure (23).

Although some preliminary screening experiments indicated that irradiation of propylene produced mutagenic products, toluene was the first hydrocarbon we studied in detail. Toluene was selected because of its predominance in urban atmospheres. It represents an important reactive component in the ambient urban hydrocarbon mix (24). It is frequently the most abundant nonmethane hydrocarbon pollutant found in populated areas, and its concentration at urban and suburban sites typically ranges from 5 to 30 ppb (25,26). In addition, many of toluene's photooxidation products (e.g., formaldehyde and peroxyacetyl nitrate [PAN]) are also produced by other simpler hydrocarbons. Finally, the photochemistry of toluene had been fairly well characterized (27).

Our experiments with toluene demonstrated that its photooxidation products were indeed mutagenic, but the product distribution proved to be too complex to permit a convenient determination of the mutagenic species. We turned our attention back to propylene, for which the photooxidation had been thoroughly studied and the major products had been identified. The chemical mechanisms for the photooxidation process had been characterized in detail (28), and they appeared to be far simpler than the mechanisms for toluene. Propylene appeared to be a good prospect for describing and understanding the formation of mutagens through photochemical processes.

The irradiations of propylene produced mutagenic products, confirming our preliminary results. In an attempt to identify the specific mutagenic compound(s), we began to change the experimental conditions. We varied the residence time (and, therefore, the product distribution) and learned that more mutagenicity was observed at long reaction times. Because ozone and NO_3/N_2O_5 are present in the system at long residence times, we studied the reaction of propylene with these compounds in separate, isolated experiments. These major subsystems of the photooxidation process could not account for the formation of the observed mutagenicity. The results led us to consider whether a major product of the photooxidation process, acetaldehyde, might react further and produce the mutagenic compound(s). We therefore conducted a series of experiments with acetaldehyde and found that it did indeed produce mutagenic products. The implications of this result are far reaching, for acetaldehyde is produced during the normal photochemical oxidation of many common pollutants and is ubiquitous in urban atmospheres at levels as high as 10-20 ppb (26,29).

Although most of the reactive hydrocarbon concentration in urban atmospheres is represented by organic compounds containing carbon and hydrogen only, a variety of chlorinated solvents are also present that are important in assessing urban exposures to air toxics (2). Many of these chlorinated hydrocarbons are considered hazardous air pollutants (HAPs) and are currently being considered for regulatory action (30) because of their potential human health effects. During the normal photochemical processes that occur in polluted atmospheres, the chlorinated HAPs may be converted to products that are more (or less) mutagenic than the reactant HAP. The mutagenicity of the chlorine-containing products can be substantially different from the products of the more common hydrocarbons. For example, chloroethylene oxide (a possible oxidation product of vinyl chloride) is 10,000 to 15,000 times more mutagenic than is ethylene oxide (31).

We conducted a series of experiments involving exposure of the Ames test bacteria Strain TA100 to the photooxidation products of allyl chloride (3-chloropropene). This particular HAP

was chosen both because it is of interest to the regulatory community and because it is the chlorinated analogue of propylene, which we also studied in detail. Our experience with propylene, along with the fact that the chemical mechanism and reaction kinetics for allyl chloride are fairly well understood (32), should facilitate the identification of individual mutagenic products for this particular HAP.

For the individual hydrocarbons studied, we irradiated mixtures of ~1 ppm hydrocarbon in the presence of ~0.5 ppm NO_X in a 22.7-m^3 Teflon smog chamber. The smog chamber can be operated in a dynamic/flowing mode, in which reactants are added continuously, resulting in a steady-state product distribution in the chamber. The product distribution is dependent upon the residence time τ (where τ = total volume/flow rate) of the gases into the chamber. With this method, a bioassay of the chamber effluent can be performed for long periods of time with a constant composition mixture of pollutants.

In urban photochemical smog systems the air toxics present exist both as gas-phase species and as particulate matter. We thought it important, therefore, to examine the distribution of mutagenic compounds between the gas and particulate phases in complex mixtures. We conducted a series of experiments in which wood smoke/NO_X mixtures were irradiated, using the same 22.7-m^3 smog chamber operated in a static mode. In these experiments the mutagenic activities of both the gas- and particulate-phase reactants and products were measured. Similar experiments were also conducted for automobile exhaust. Information regarding the extent to which mutagenic compounds can be produced from irradiation of automobile exhaust/NO_X mixtures is clearly needed, given the importance of automobile exhaust as a source of reactive hydrocarbons that control urban photochemical smog chemistry.

It has also recently been demonstrated that NO_3/N_2O_5 mixtures can react with gas-phase species such as naphthalene (33), a wood stove emission component, to produce mutagenic products (i.e., nitronaphthalenes). We also conducted an experiment in which we reacted NO_3/N_2O_5 with propylene and also one in which we reacted NO_3/N_2O_5 with a wood smoke mixture. The mutagenic activity of the gas- and particulate-phase (for wood smoke) reaction products was measured.

The results of a preliminary laboratory study indicated that PAN is one major mutagenic product of the photooxidations of toluene, acetaldehyde, and propylene. We therefore conducted a detailed investigation of the mutagenic activity of PAN and several other peroxyacyl nitrates,

specifically, peroxypropionyl nitrate (PPN), peroxybutyryl nitrate (PBN), and peroxybenzoyl nitrate (PBzN).

The results of all experiments described in this report will be discussed in terms of the nature of the photochemical processes that produce the mutagenic products and in terms of the specific photooxidation products that contribute significantly to the observed mutagenic activities. We will also attempt to determine the relative potential contribution to atmospheric mutagen production for each of the individual hydrocarbons studied and, to some extent, for the complex mixtures studied.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The experiments described in this report indicate that mutagenic compounds (as determined by using the Ames test) are produced as a result of atmospheric photochemistry. For many HC/NO_X systems studied, including propylene and toluene, the photooxidation products were much more mutagenic than the reactant hydrocarbons. Not all HC/NO_X irradiations produced mutagenic products, however. The photooxidation products of allyl chloride, a chlorinated HAP, were shown to be extremely mutagenic. Some HAPs may be important in terms of atmospheric mutagenesis, even though their ambient concentrations are relatively low. For complex reactive mixtures such as wood smoke, irradiation can produce substantial increases in the mutagenic activities of both gas- and particulate-phase species. It was found that the majority of the mutagenic activity of the product mixture of wood smoke is associated with gas-phase species. Much of the increase in mutagenic activity for the particulate phase in our experiments is probably the result of adsorption of gas-phase (low volatility) mutagenic products onto existing particulate matter, rather than a reflection of reactions occurring on the surface of the particulate matter.

For most of the product mixtures investigated, a significant portion of the mutagenic activity observed may have been caused by PAN, which has been shown to be mutagenic. The absolute value of the mutagenic activity of this species is currently uncertain, however, and much more work is necessary to determine the mutagenic potential of PAN at ambient concentration levels. Because PAN is ubiquitously present in polluted urban atmospheres, it would also be desirable to determine the biological impact of PAN on other biological systems by using additional bioassay techniques.

At this point, laboratory experiments have shown that pollutants commonly found in urban air can be transformed into mutagenic products as a result of typical urban photochemical processes. These results suggest the need to extend this effort in both laboratory and field measurement areas. Ambient measurements of mutagenic activities in urban air masses would be helpful in determining the possible correlation of mutagenic activity of the air mass with the presence of photochemical oxidants such as PAN, and may aid in the overall assessment of the contribution of urban photochemistry to the presence of atmospheric mutagens.

SECTION 3

EXPERIMENTAL PROCEDURES

A schematic diagram of the reaction chamber and exposure chamber system is shown in Figure 3-1. The reaction chamber is a cylindrical Teflon bag surrounded by lights to simulate the solar spectrum at the earth's surface. The chamber can be operated in two distinct modes: static and dynamic. In the static mode, the chamber is first brought to the desired initial pollutant concentrations. Then, the lights are turned on, and the concentrations of the pollutants are monitored as a function of time. (Figure 4.1-1 is an example of a concentration vs. time profile obtained from a static-mode irradiation.) In the dynamic mode, the chamber can be operated as a continuously stirred tank reactor, with reactants being continuously added to the irradiated chamber. The extent of reaction in the dynamic mode is controlled by the residence time. The residence time is the average time that a molecule remains in the chamber and is calculated by dividing the volume of the chamber by the flow rate through the chamber. At short residence time, the product distribution is similar to that present at short reaction during the comparable static-mode irradiation. Similarly, at long residence time, the products are like those found near the end of the static-mode experiment. Because the product distribution for the dynamic mode is determined by the residence time and not by how long the lights have been on, a consistent, unchanging product distribution can be maintained for whatever period of time is necessary to conduct chemical and biological analyses. (Figure 4.1-2 is an example of a concentration vs. time profile of a dynamic-mode irradiation.)

Clean air was produced by an AADCO clean air generator supplied with compressed air from a Quincey Model 325-15 air compressor. The dilution air from the AADCO was controlled in the 0-5 ft^3/min range with a Teledyne Hastings-Raydist Model NAHL-5P mass flow controller. For the toluene experiments the air was humidified by a Sonimist Model 600L ultrasonic spray nozzle. For the dynamic-mode experiments the reactants were allowed to flow into the chamber at 2 to 5 ft^3/min, and they were mixed with a 60.5-cm diameter three-blade impeller powered with a 0.25-hp motor. The chamber was constructed of a 7.5-m-long cylindrical Teflon bag connected on each end to aluminum end plates, 1.96 m in diameter and coated with fluorocarbon paint. Irradiation of the reactor was provided by a total of 180 GE F-40 blacklight bulbs and 36 sunlamps. For experiments with the individual hydrocarbon/NO_X mixtures, in which the chamber is operated in a dynamic mode, the reactants are mixed in a 150-L stainless steel inlet manifold and then transferred to the chamber through the end plate. Toluene (Fisher Scientific, HPLC grade) was

added to the inlet manifold by bubbling N_2 (MG Scientific, prepurified grade) through an impinger bottle containing ~100 ml toluene maintained at 0°C. Flow through the impinger was controlled with a Tylan Model FC260 (0-20 cm^3/min) mass flow controller. One percent mixtures in nitrogen of acetaldehyde, propylene, allyl chloride, and nitric oxide (NO) were similarly added (MG Scientific) by using a mass flow controller. As shown in Figure 3-1, N_2O_5 was prepared by mixing O_3 and NO_2 (1% in N_2) in a 3-L Pyrex mixing bulb. The O_3 was produced at ~1% in O_2 with a Welsbach Model T-408 O_3 generator supplied with zero-grade O_2. The O_3 reacted with NO_2 in the mixing bulb to produce NO_3, which was in equilibrium with N_2O_5, as indicated in Reactions 1 and 2. The resultant N_2O_5/NO_2 mixture was then diluted with clean dry air in the inlet manifold.

$$NO_2 + O_3 \rightarrow NO_3 + O_2 \qquad 1$$

$$NO_3 + NO_2 \rightleftharpoons N_2O_5 \qquad 2,\text{-}2$$

For the wood smoke/N_2O_5 experiment the initial N_2O_5 concentration was ~2 ppm, and for the propylene + N_2O_5 experiment, it was ~1.8 ppm.

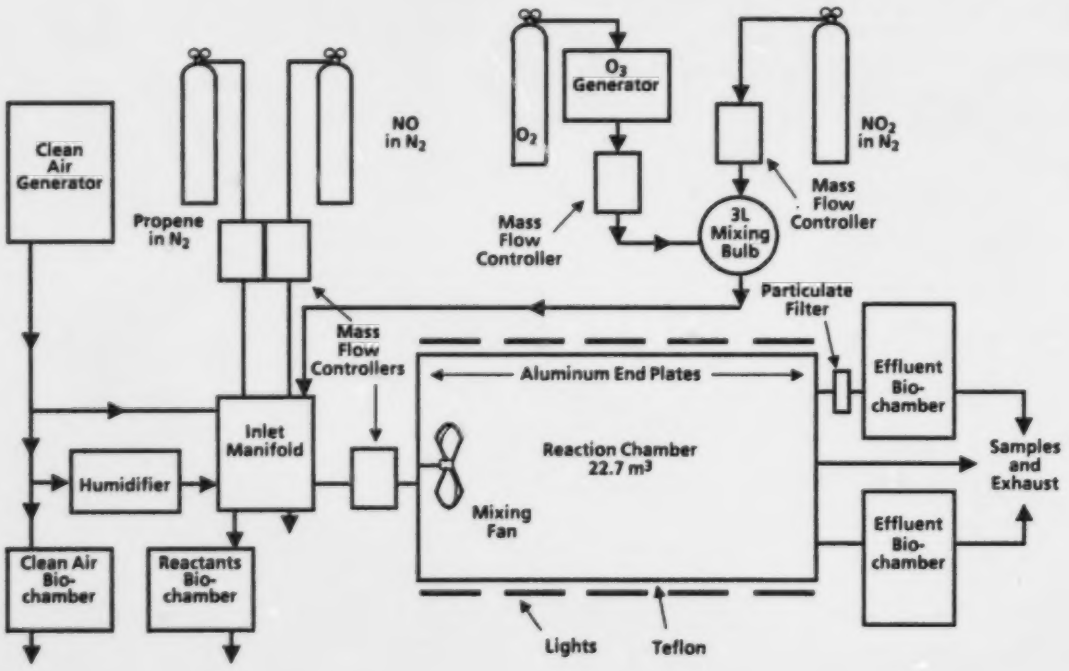

Figure 3-1. Schematic diagram of the reaction chamber apparatus.

For the wood smoke experiments, wood combustion was achieved by using an Ashley Model 7150B wood stove. The wood used was a mixture of locally obtained varieties of oak. To load the reaction chamber, a portion of the wood smoke from the chimney was continuously drawn through an 8-cm steel pipe to a dilution tunnel where the wood smoke was diluted with ambient air and cooled to ambient temperature. The unfiltered ambient air was drawn through the dilution tunnel by a 0- to 20-m^3/min turbine. This dilute wood smoke mixture was then added to the reaction chamber by using a Metal Bellows Corporation Model MB-151 metal bellows pump. Similarly, in the experiments involving automobile exhaust, the mixture was transferred to the chamber from the tail pipe with a metal bellows pump. A 1980 Toyota Corolla (catalyst equipped) operating at idle at relatively high engine rpm was the source. The fuel used was a "super unleaded" grade ([R + M]/2 = 91). For the wood smoke and automobile exhaust experiments the initial total hydrocarbon concentrations were ~20 and 12 ppmC, respectively. In both cases the initial NO_x concentration was ~0.7 ppm.

The PAN-type compounds were prepared by several different techniques. For PAN itself, a number of experiments were conducted in which PAN was synthesized in the gas phase by irradiation of $Cl_2/NO_2/CH_3CHO$ mixtures (34), as indicated in Reactions 4-6 below.

$$Cl_2 + h\nu \rightarrow 2\,Cl \qquad 4$$

$$Cl + CH_3CHO \xrightarrow{O_2} HCl + CH_3C(O)O_2 \qquad 5$$

$$CH_3C(O)O_2 + NO_2 \rightleftharpoons CH_3C(O)O_2NO_2 \ (PAN) \qquad 6,\text{-}6$$

The PAN was then collected in a cold trap and vacuum distilled from –63°C to –110°C. PAN, PPN, and PBN were also prepared in dodecane solution by nitration of the corresponding peroxy-acid (35,36). The PAN-type compounds were also produced photochemically with the reaction chamber operating in a dynamic mode, using mixtures of O_3, NO_2, and the corresponding aldehyde, as shown in Reactions 7-9 below for PBzN.

$$O_3 + NO_2 \rightarrow NO_3 + O_2 \qquad 7$$

$$NO_3 + C_6H_5CHO \xrightarrow{O_2} HNO_3 + C_6H_5C(O)O_2 \qquad 8$$

$$C_6H_5C(O)O_2 + NO_2 \rightleftharpoons C_6H_5C(O)O_2NO_2 \qquad 9,\text{-}9$$

Four 190-L Teflon-coated exposure chambers were used for exposure of the bacteria to the various air mixtures studied. The bacteria were exposed to the following different types of air masses: reactants, filtered or unfiltered products, clean air, and, for the wood smoke experiments, ambient air. The gases were transferred to each of the exposure chambers through 3/8-in. Teflon tubing at a flow rate of 14 L/min, which was maintained and measured with a needle valve and a calibrated rotameter. All lines were as short as possible to maximize transfer efficiency.

The experiments for measuring gas-phase mutagenic activities were conducted by exposing the Ames test bacteria *Salmonella typhimurium* to the effluent from the smog chamber. Strains TA100 and TA98 were used, both with and without S9 metabolic activation. The exposures were conducted by allowing the chamber air to flow (at 14 L/min) through Teflon-coated, 190-L exposure chambers (essentially glove boxes) loaded with, typically, 50 covered Pyrex Petri plates containing the bacteria in a nutrient agar. In these experiments, the plates were dosed with the components of the chamber air by uncovering them for a specific period of time. This allowed the agar-soluble species to deposit into the test plates as the air mass flowed through the exposure chamber. Because the agar is mostly water, those species that are water soluble (i.e., polar), as are most photooxidation products, deposit into the test plates. Many polar species, such as formaldehyde, are completely removed from the air mixture as it flows through the exposure chambers (see Section 4.1).

Glass Petri dishes containing *S. typhimurium* were provided by the Health Effects Research Laboratory of U.S. EPA. The plates were prepared by adding 0.1 ml of the *S. typhimurium* culture to 3 ml of an agar overlay at 45°C (with or without 0.5 ml of S9 mix). This mixture was then poured onto ~45 ml of plate agar in a glass Petri dish. Colony counting was performed with an Artec 880 automatic colony counter, using previously published guidelines (37). The test procedures used were those of Ames *et al.* (22), except for the following modifications: [1] glass Petri dishes were used, [2] 45 ml of base agar per plate was used, [3] minimal histidine at the same final concentration was placed in the bottom agar rather than the top agar, and [4] 3 ml of overlay agar with $\sim 1 \times 10^8$ bacteria were used. The rat-liver homogenate (S9) fraction was prepared from male Charles River CD-1 rats (Wilmington, MO) induced with Aroclor 1254 (22). In many of the experiments the exposure chambers were also loaded with several "survivor" plates. For the survivor plates, the bacteria concentration was diluted by roughly 10^4 and additional histidine was added. The magnitude of the dilution was such that, in the absence of toxicity, ~500 colonies per plate were produced. Therefore, these plates could be used to indicate the possibility of toxicity effects for the test plates.

For several experiments, plates containing either distilled buffered water or water containing 2 mM NaOH (to measure PAN deposition) were added to the exposure chambers as a model for the quantity of gas-phase component that solubilized into the biotesting medium. For the toluene experiments the acid and base/neutral fractions of the water were extracted with methylene chloride after each exposure (38). The extracts were then analyzed for benzaldehyde and the cresols by GC/MS. For several experiments the water plates were analyzed for formaldehyde by the chromotropic acid technique (39), and for nitrite, nitrate, formate, and acetate ions by ion chromatography. PAN deposition was measured by using the NaOH plates. In basic media, PAN decomposes as indicated in Reaction 10, enabling determination of its concentration by measurement of acetate or nitrite ions (40).

$$CH_3C(O)OONO_2 + 2OH^- \rightarrow CH_3C(O)O^- + NO_2^- + O_2 + H_2O \qquad 10$$

For the wood smoke and automobile exhaust experiments, particulate matter was removed from the reactants effluent, and ambient air streams by Teflon-impregnated glass-fiber filters (T60A20 Pallflex, 13.34 cm). The particulate-phase filter samples were Soxhlet extracted with 250 ml of pesticide-grade methylene chloride (Fisher Scientific) for 6 h. The mutagenic activities of these concentrated extracts were then measured by using the standard plate incorporation test (22).

For each gas-phase exposure, the gas-phase mixture was allowed to flush completely through the exposure chamber several times, at which point the plates were uncovered, effectively starting the exposure. Then, groups of plates were covered periodically throughout the exposure period, effectively stopping the dosage of the plates at certain points and enabling construction of dose-response curves. For these experiments the plates were exposed for a period of time (typically 2-20 h) necessary to induce a significant increase in the reversion level. After the exposures, the plates were incubated at 37°C for 48 h and the number of revertants/plate were counted.

For some experiments (e.g., wood smoke and automobile exhaust), it was not feasible to operate the chamber in a dynamic mode. In those cases, the exposures were conducted by first preparing the test mixture in the reaction chamber and then exposing the bacteria to the air sampled from the reaction chamber. Air withdrawn from the chamber was continuously replaced by clean dilution air, which diluted the test mixture. For these experiments the effective exposure times (i.e., dilution-corrected) for the dose-response curves were obtained by using Expression I:

$$t_{eff} = \int_o^{t_{ex}} \exp(-kt)dt \qquad (I)$$

where t_{ex} is the exposure time in real time and k is the dilution rate constant.

All experiments began with a conventional static-mode smog chamber irradiation, so that the temporal variation of reactant and product concentrations could be determined and the desired extent of reaction for the dynamic experiments could be chosen. By varying the reaction chamber residence time in the dynamic-mode experiments, we were able to conduct bioassay measurements for steady-state mixtures having product distributions corresponding to different regions of a conventional static-mode irradiation. Because the concentration profiles for many photooxidation products (e.g., organic peroxides) are highly dependent on the extent of reaction (e.g., presence or absence of NO), the change in the mutagenic activity of the mixture as the reaction proceeds can be interpreted in terms of changes in the product distribution. For several experiments conducted with individual hydrocarbons, exposures were conducted at short reaction chamber residence time ($\tau \simeq 3$ h), where NO is present, and at long residence time ($\tau \simeq 7$ h) near the ozone and PAN maxima. When NO is present, formation of peroxides is inhibited, whereas at long residence time when NO has been removed, peroxides such as PAN can be present in significant concentrations.

Throughout the static-mode irradiations and the dynamic-mode exposures, a variety of gas- and particulate-phase species were measured from both the reaction chamber (at the end opposite the inlet manifold) and the exposure chambers. Toluene was measured by using a Varian Model 1400 GC containing a glass column packed with 0.1% SP-1000 on Carbopack C, operated at an He flow rate of 20 cm^3/min and a temperature of 200°C. Propylene was measured by using a Hewlett-Packard Model 5840A GC containing a 6.4-mm x 2-m stainless steel column packed with 80/100 Porapak QA and operated isothermally at 130°C. Acetaldehyde was also measured by operating the Porapak QA column at 145°C. Allyl chloride was measured by using the Varian GC containing a 3.2-mm x 2-m glass column packed with 1% SP-1000 or Carbopack B at 80°C. Hydrocarbons were injected by using a solenoid-activated Seizcor six-port valve that was switched on and off with a Chrontrol Model CD timer. Calibrations were performed by preparing ~1-ppm standards in clean air in 200-L Teflon bags. NO and NO_X were measured by using a CSI Model 1600 oxides-of-nitrogen analyzer calibrated with a certified standard of NO in N_2 (MG Scientific). Ozone was measured with a Bendix Model 8002 ozone analyzer calibrated by using a Dasibi Environmental Corporation UV ozone monitor. Relative humidity in the chamber was measured with an EG + G Model 880 dew point hygrometer calibrated with saturated salt solutions. Particle number concentrations were measured with a TSI Model 3020 condensation nuclei counter. Aerosol size distributions (0.01-1 μm) were measured with a Thermo Systems, Inc., Model 3030 electrical aerosol analyzer (EAA). For the wood smoke and automobile exhaust experiments the total hydrocarbon

concentration was determined by using a Beckman Model 400 HC analyzer, which was calibrated with pure samples of propane. CO was measured by using a Bendix Model 8501-5CA infrared CO analyzer.

The PANs were measured by using a Valco electron capture detector after separation on a column, which was packed with 10% Carbowax 400 on Gaschrom Z, at 25°C. For several experiments this chromatograph was calibrated according to the procedure of Lonneman *et al.* (41). For some experiments PAN and chloroperoxyacetyl nitrate (CPAN) were measured by bubbling 30-L chamber samples through 5 cm^3 of deionized water adjusted to pH 12 with NaOH, which converts these species to acetate or chloroacetate ions, respectively (40). The ions were then separated and detected by using 10^{-3} M HCl eluent with a Dionex System 10 ion chromatograph containing a Dionex HPICE AS2 column. For the series of experiments in which we measured the mutagenic activity of several PANs (Section 4.6), the PAN chromatograph was calibrated by preparing samples of the pure, synthesized PAN (35) in air in Teflon bags. The PAN concentration was measured as the total NO_X concentration by using the NO_X monitor. We determined that the efficiency of the monitor for converting PAN to NO_X is 100%. Methyl nitrate was also measured by using the PAN chromatograph, which was calibrated with pure samples synthesized according to the procedure of Johnson (42).

The aldehydes, formaldehyde, acetaldehyde, acrolein, glyoxal, methylglyoxal, and benzaldehyde were measured by using the DNPH/HPLC technique of Kuntz *et al.* (43). Benzaldehyde and *o*-,*m*-, and *p*-cresol analyses were also conducted by injection of 5-L cryotrapped samples into an HP 5985 GC/MS containing a 2-m x 2-mm glass column packed with 0.1% SP-1000 on 80/100 Carbopack C. Nitric acid was measured by drawing air through a 25-mm nylon filter (1-μm pore size), extraction of the filter with 10^{-5} M perchloric acid solution, and subsequent analysis for nitrate ion by using a Dionex System 12 ion chromatograph with a Dionex Model 60361 anion separator column.

For the propylene experiments (Section 4.2) the organic nitrates (PGDN, 2HPN, 2NPA) were measured on a Varian 1200 GC, employing a 6.4-mm x 2-m glass column packed with 10% SP-1000 on 80/100 Supelcoport and operated isothermally at 155°C. Detection was achieved with a Valco Model 140B electron capture detector. Samples were injected with a 5-ml glass and Teflon syringe. These products were identified under the same conditions by using an HP Model 5985 GC/MS. Details of the product identification and the calibration procedure are in a separate publication (44). Weak acids (e.g., HCOOH) were collected by bubbling the effluent through a 1 mM NaOH solution.

Ion chromatography employing a 2.5 mM $B_4O_9^{2-}$ eluent was used for separation and detection of the weak acid anions. Calibrations were made with solutions prepared from the alkali salts.

The allyl chloride photooxidation products chloroacetaldehyde, 3-chloroacrolein, and 1,3-dichloroacetone were collected by bubbling 10-L chamber samples through 2 cm^3 of methanol cooled to 0°C in an impinger bottle. Collection efficiency for this technique was found to be ≥99%, as determined by using two bubblers in series. These three compounds were separated by a 3.2-mm i.d. glass column, packed with 1% SP-1000 on Carbopack B, that was contained in a Varian 1200 GC. Detection was achieved with a Valco Model 140B electron capture detector. The identification of these species was conducted by GC/MS, as described in a previous publication (32).

An ethylene/NO_X dynamic-mode exposure was conducted to test for the mutagenic activity of a subset of the photooxidation products, specifically the inorganic species HNO_3, O_3, H_2O_2, and HO_2NO_2. Ethylene was measured by using a Varian 1400 GC (FID), which contained a 3.2-mm x 2-m stainless steel column packed with 60/80 Carbosieve G and operated at 175°C.

For the wood smoke and automobile exhaust experiments, methane, acetylene, ethylene, and ethane were measured with a Varian 1400 GC, using a 3.2-mm x 2-m stainless steel column packed with 60/80 Carbosieve G and operated at 150°C. Chloromethane, propylene, propane, and 1-butene were measured with a Varian 1200 GC, using a 6.4-mm x 2-m stainless steel column packed with 80/100 Porapak QS and operated at 130°C. Isoprene, furan, and 2-methyl furan were measured with an HP Model 5840 GC, using a 2-mm x 2-m glass column packed with 0.1% SP-1000 on 80/100 Carbopack C and temperature programmed from 40 to 200°C at 20°C/min. Benzene, toluene, xylenes, benzaldehyde, styrene, and *m*-methyl styrene were measured by pumping 25-L samples through Pyrex tubes packed with Tenax GC. The Tenax-filled tubes were then thermally desorbed at 275°C with a Nutech 320 thermal desorption unit. The desorbed samples were analyzed with an HP 5985 GC/MS, which contained a 2-mm x 2-m glass column packed with 10% SP-1000 on 80/100 Supelcoport. The column was temperature programmed from 50 to 225°C at 20°C/min. Calibrations were performed by desorption of Tenax tubes containing samples of the pure compounds at the 1-nmol level.

The particulate-phase filter samples were Soxhlet extracted with 250 ml of pesticide-grade methylene chloride (Fisher Scientific) for 6 h. The extracts were then concentrated under a stream of prepurified N_2 (MG Scientific) and analyzed for PAH concentration, using the GC/MS, by injection onto a 2-mm x 2-m glass column packed with 3% SP-2250 on 80/100 Supelcoport. The column was programmed from 200 to 300°C at 10°C/min. For the compounds measured, the GC/MS peak areas

were measured relative to pyrene and quantified as pyrene. The extracts were prepared for bioassay by solvent exchanging a portion of the methylene chloride extract with 2-ml samples of dimethyl sulfoxide (Burdick and Jackson Laboratories, Inc.). Blank filters were also extracted to serve as both chemical and bioassay controls.

To determine the effectiveness of the use of XAD-2 as a collection medium for subsequent measurement of gas-phase mutagenicities, a wood smoke/NO_x irradiation was conducted in which gas-phase mutagens were collected by pumping the filtered reaction chamber air through an 11-cm x 8.5-cm diameter stainless steel cylinder packed with XAD-2 resin. After exiting this XAD-2 trap the air then passed into an exposure chamber. After the exposure period the XAD-2 was Soxhlet extracted with methylene chloride, followed by extraction with methanol. Each of the extracts were then concentrated, using a kuderna-danish concentrator, and then solvent exchanged with DMSO, followed by the standard plate incorporation test.

SECTION 4

RESULTS

4.1 IRRADIATED TOLUENE/NO_X MIXTURES

For irradiated toluene/NO_X mixtures, the consumption of toluene occurs solely through reaction with OH radicals. Static toluene/NO_X irradiations were first performed to determine the product distributions as a function of irradiation time. Figure 4.1-1 shows the results for a static (i.e., no flow) irradiation of 970 ppb toluene and 390 ppb NO, along with the time profiles for several major components of interest. As can be seen in this figure, a sharp burst of particle formation occurred at or near the ozone maximum. We have found the overall reaction rate to be heavily dependent on the chamber humidity, possibly due to heterogeneous production (45) of nitrous acid (HONO), which can photolyze to produce OH radicals. We therefore attempted to operate the chamber at ~50% relative humidity (at 22°C) in all experiments. The relative humidity for the static experiment was 57% at 19°C.

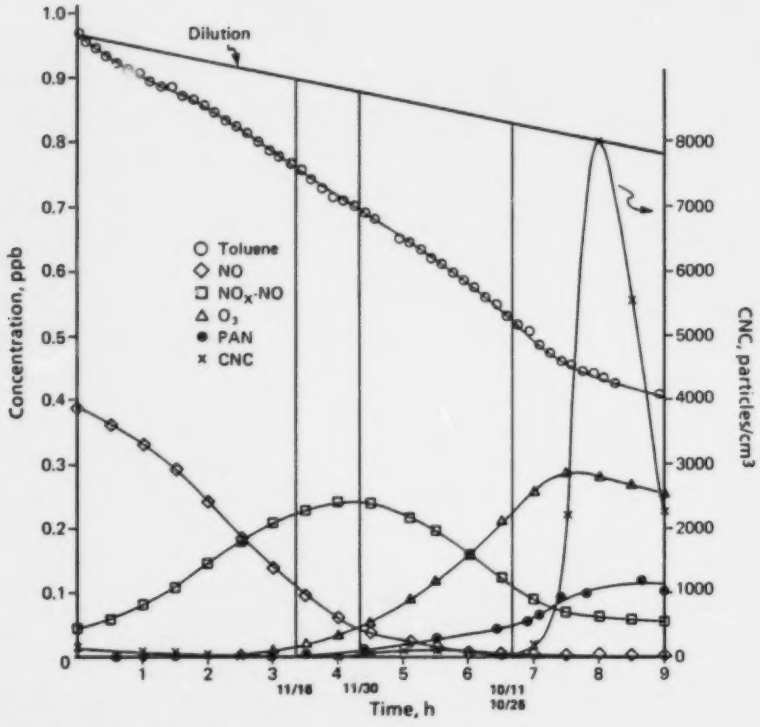

Figure 4.1-1. Static-mode toluene/NO_X/H_2O/air irradiation.

For the dynamic runs, we attempted to maximize the compositional differences between the two sets of exposures. For the toluene study, duplicate exposures were conducted at both short extent of reaction (i.e., with NO present) and at long extent of reaction (i.e., near the ozone maximum). We were, however, limited on the fast-flow (i.e., small τ) end by the maximum output of the clean air generator (5 ft^3/min or τ = 2.7 h) and on the slow-flow (large τ) end by the required sampling and exposure chamber feed rates (2 ft^3/min or τ = 6.7 h). For the dynamic exposures, residence times (τ) of 3.0 h and 6.7 h were chosen. Because of the dependence of the reaction rate on humidity and apparent wall radical sources, the extent of reaction at these times was difficult to duplicate exactly. Figure 4.1-1 shows, by experiment date, the extent of reaction (by the vertical lines) at which the bioassays were performed, based on the observed distribution of toluene, NO, NO_X-NO, and ozone. The 11/16 and 11/30 and the 10/11 and 10/26 experiments were meant to be duplicates of each other. Each of the four experiments were conducted at the same nominal reactant concentrations, as indicated in Figure 4.1-1.

The results of the biotesting for these four experiments are presented in Table 4.1-1. The "spontaneous" samples are a group of plates prepared and counted in the same way as the exposed plates, but which remain in the biotesting laboratory throughout the exposure. The spontaneous plates thus measure the natural reversion rate observed under sterile conditions. Although the data for the clean air biochamber are, on the average, larger than the data for the spontaneous plates, we do not interpret this as being a positive response to clean air. Rather, we attribute this to exposure of the clean air plates to a number of environmental factors (such as a brief exposure to sunlight) that the spontaneous plates do not experience. For the purposes of interpreting the data for the other exposure chambers, it is reasonable to compare the numbers to those for the clean air biochamber.

In addition, two clean air irradiations were performed at τ = 3.0 h and τ = 6.7 h. The bacteria were exposed to the irradiated clean air, but no significant increase in the revertant level for the irradiated clean air, relative to the nonirradiated air, was observed for either residence time. Therefore, we concluded that irradiation of clean air does not produce gas-phase mutagens.

The following products were measured in these experiments: PAN, ozone, formaldehyde, acetaldehyde, glyoxal, methylglyoxal, benzaldehyde, the three cresol isomers, nitric acid, carbon monoxide, and total particulate matter. The average effluent concentrations for each species are presented in Table 4.1-2. These concentrations agreed for the duplicate experiments to within ±20%.

TABLE 4.1-1. BIOASSAY RESULTS, TOLUENE/NO_X EXPERIMENTS, AVERAGE REVERTANTS/PLATE (S.D.)*

Exposure†	11/17 and 11/30; τ = 3.0 h								10/11 and 10/26; τ = 6.7 h							
	TA100		TA100 + S9		TA98		TA98 + S9		TA100		TA100 + S9		TA98		TA98 + S9	
S	119	(14)	117	(5)	25	(5)	31	(5)	124	(19)	119	(16)	--		--	
	117	(16)	117	(12)	48	(4)	50	(4)	154	(15)	146	(19)	31	(5)	40	
BCA	162	(23)	160	(35)	39	(6)	52	(5)	213	(23)	219	(44)	--		--	
	263	(39)	280	(47)	81	(15)	88	(13)	158	(23)	179	(18)	21	(9)	16	(4)
BR	181	(26)	171	(26)	30	(4)	51	(6)	321	(40)	380	(24)	--		--	
	222	(40)	250	(45)	--		72	(12)	242	(44)	249	(31)	24	(10)	49	(7)
BF	242	(23)	209	(37)	28	(6)	44	(9)	689	(27)	639	(106)	--		--	--
	308	(50)	286	(31)	75	(11)	81	(10)	751	(109)	548	(177)	63	(22)	91	(10)
BU	211	(30)	189	(24)	31	(5)	45	(6)	705	(44)	583	(52)	--		--	--
	285	(41)	274	(37)	67	(7)	78	(12)	863	(112)	636	(81)	76	(8)	80	(11)

* First and second lines show 11/17 (or 10/11) and 11/30 (or 10/26) results, respectively.
† S = spontaneous; BCA = clean air; BR = reactants; BF = filtered effluent; BU = unfiltered effluent.

TABLE 4.1-2. AVERAGE (± 20%) PRODUCT CONCENTRATIONS, TOLUENE/NO_x IRRADIATIONS

	Effluent, ppb		Test Plates,* nmol/plate	
	τ = 3.0	τ = 6.7	τ = 3.0	τ = 6.7
PAN	14	132	91	198
O_3	48	280	–	–
HCHO	48	82	367	933
CH_3CHO	5	8	–	–
CHOCHO	16	24	34	103
$CH_3C(O)CHO$	20	34	125	333
C_6H_5CHO	7	12	38	66
o-cresol	11	5	111	30
m-cresol	3.5	1.0	37	6
p-cresol	3.5	1.5	37	9
HNO_3	62	84	–	–
CO	169	490	–	–
Particles/cm^3	0	400	–	–

* Calculated from Expression II.

In Figure 4.1-2 we present the reactant profiles for the 10/26 (τ = 6.7 h) experiment, which demonstrate the approach to steady state and the degree to which it was maintained in these experiments. Once the effluent reactant and product distributions reached steady state, the four biochambers were loaded with the covered Petri plates. The biochamber product concentrations were then allowed to come back up to their steady-state values. At this point the various product concentrations were measured in the biochambers with the plates covered. The values obtained thus represented the transfer efficiencies between the reaction chamber and the exposure chambers. These measurements required an ~2-h period before the plates could be uncovered. At the end of this 2-h period the plates were uncovered and the exposure was begun. During the ~18.5-h exposures the product concentrations were measured in the chamber effluent and in the biochambers. From the differences in biochamber concentrations before and after the plates were uncovered, we can determine the relative amounts of exposure to each chemical (see Discussion).

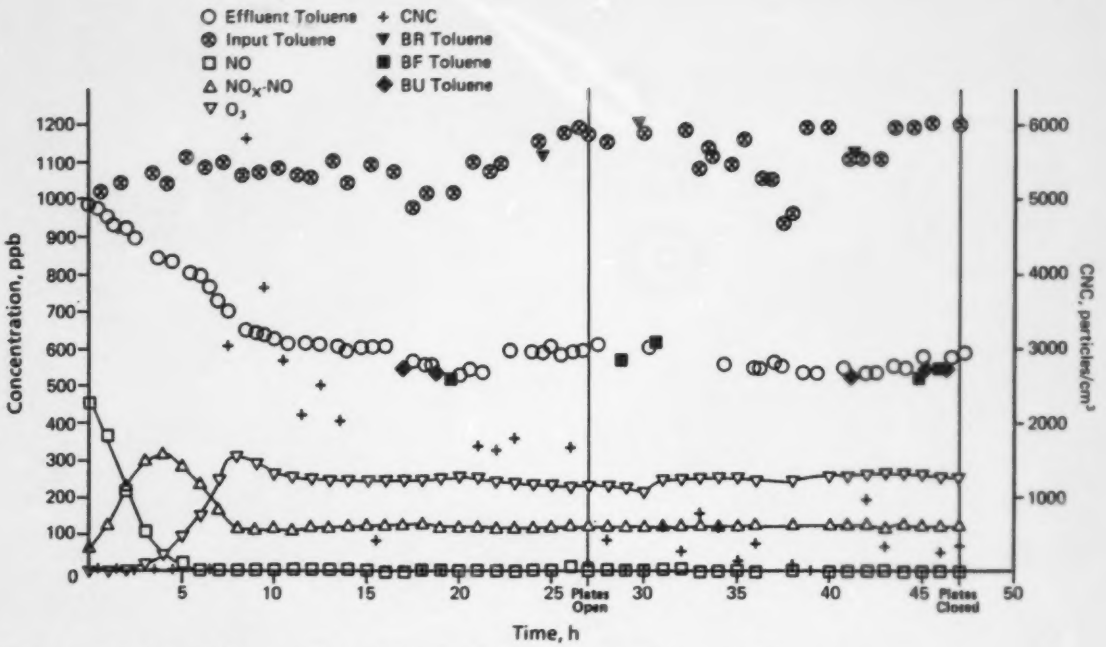

Figure 4.1-2. Reaction profile: toluene/NO_X irradiation, 10/26, τ = 6.7 h.

In Figures 4.1-3 – 4.1-5 we present reaction and exposure chamber measurements for PAN, formaldehyde, and methylglyoxal, respectively. PAN is only slightly water soluble; only ~15% of the PAN passing through the exposure chamber deposits into the plates. For very water soluble species such as formaldehyde and methylglyoxal, essentially all of the gas-phase components deposit into the plates when they are opened. A similar measurement could not be done for acetaldehyde, since it is emitted from the plates when they are opened, possibly as a metabolic product from the bacteria. For all but glyoxal, the transfer efficiencies of the various products from the reaction chamber to the biochambers were at least 50%.

As a model for determining the amount of vapor-phase material deposited into the plates, four plates containing buffered water (phosphate/biphosphate buffer, pH = 7.4) and one with pure deionized water were placed in each biochamber during the exposure period. The buffered water is used as the best model of the agar that can also be easily analyzed. Deionized water is used to circumvent ion chromatographic analysis problems. The concentrations found in the water plates can then be compared with the calculated deposition into the bioassay medium (see Discussion). This was done for the 10/26, τ = 6.7-h run for the cresols, benzaldehyde, formaldehyde, nitrite, and nitrate. The cresols, benzaldehyde, and formaldehyde were measured in the buffered water plates, and nitrite and nitrate were measured in the deionized water plates. The results are presented in Table 4.1-3.

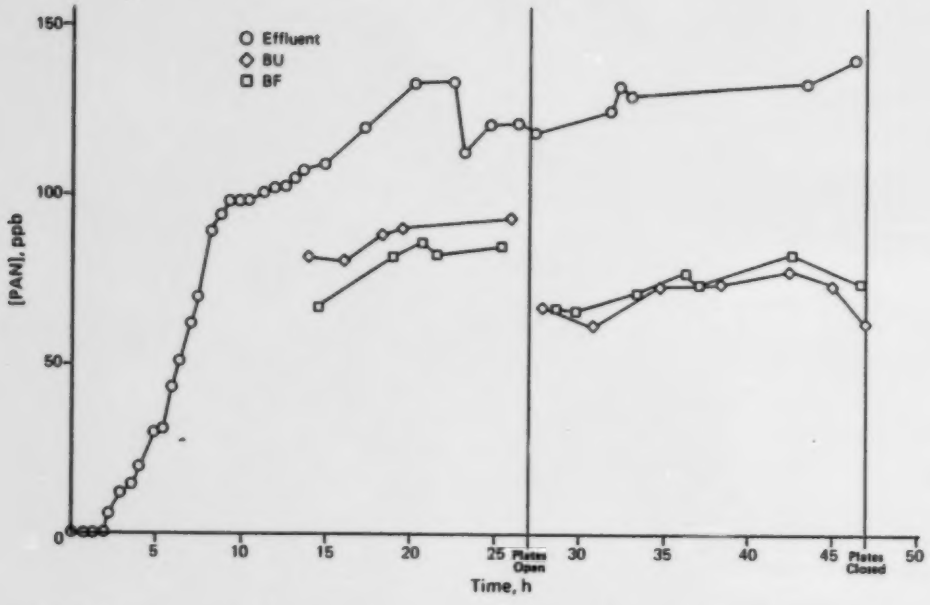

Figure 4.1-3. PAN concentrations: toluene/NO_X irradiation, 10/26, τ = 6.7 h.

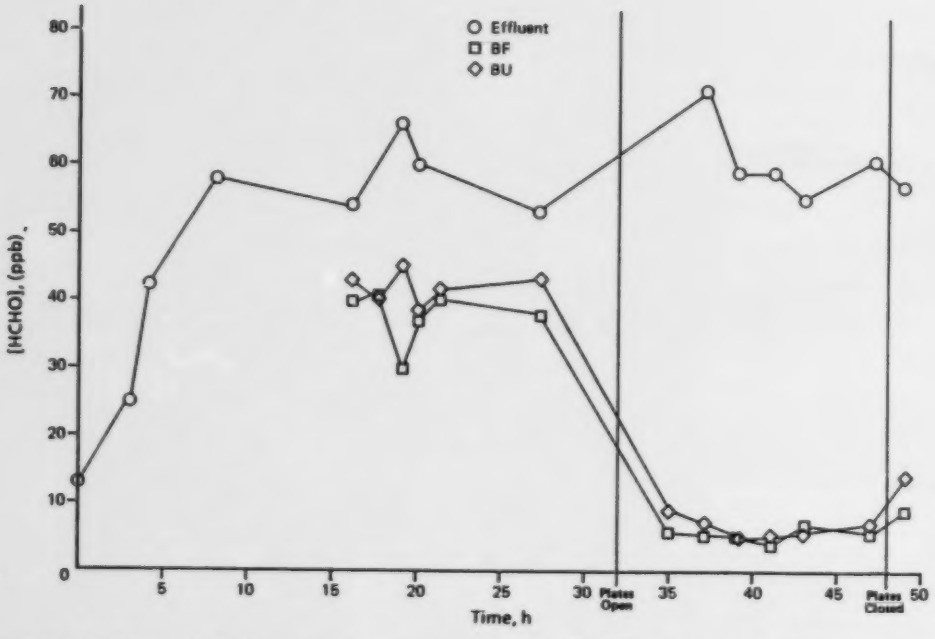

Figure 4.1-4. Formaldehyde concentrations: toluene/NO_X irradiation, 11/30, τ = 3.0 h.

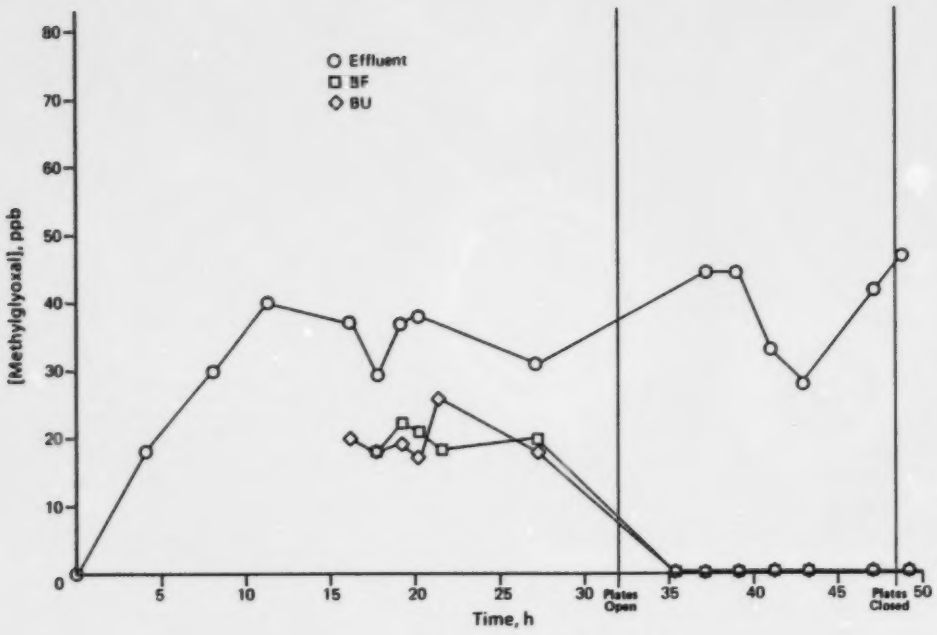

Figure 4.1-5. Methylglyoxal profiles: toluene/NO_X irradiation, 11/30, τ = 3.0 h.

TABLE 4.1-3. WATER PLATE CONCENTRATIONS, NANOMOLES PER PLATE (10/26, τ = 6.7 h, TOLUENE/NO_X IRRADIATION)

Exposure	C_6H_5CHO	*o*-Cresol	*m*-Cresol	*p*-Cresol	HCHO	NO_2^-	NO_3^-
BCA	<1	<1	<1	<1	43	87	32
BR	<1	<1	<1	<1	130	456	16
BU	5	17	6	8	600	348	210
BF	5	11	3	4	613	261	32

It is clear from inspection of the data in Table 4.1-1 that there is a significant difference in results between the two residence times for the effluent biochambers. Whereas there appears to be no measurable increase in revertants for the effluent-exposed biochambers at τ = 3.0 h (relative to the clean air biochambers [BCA]), there is a definitely significant increase for the filtered and unfiltered biochambers (BF and BU) at τ = 6.7 h (roughly 500 induced revertants per plate for TA100,

relative to the clean air plates). The effect appears to be of the same relative order of magnitude for both TA100 and TA98, indicating the possibility of more than one type of mutagen being present at this residence time. (Mutations occur by different mechanisms in the two strains. [22]) In addition, the number of revertants appears to be the same with and without S9 activation, indicating that the mutagens present are direct acting. Although there is a measurable concentration of aerosol present at τ = 6.7 h, the fact that the BF and BU responses were comparable indicates that particulate matter does not contribute significantly to the observed response. It is quite likely that the extent of deposition of particulate matter into the plates in BU is very low.

After these initial toluene experiments, a problem with the clean air bioassay plates was identified. The clean air plates were being exposed to sunlight during transport of the plates to the NSI-ES facility, resulting in an elevated level of mutagenicity. In addition, we found that dose-response curves could be obtained by covering groups of plates throughout the exposure. Therefore, in 8/86, an additional toluene/NO_X dynamic experiment was conducted at shorter residence time (τ = 2.7 h) for an irradiated mixture of 5.1 ppm toluene/0.9 ppm NO_X. Because the HC/NO_X ratio was much higher for this experiment, the light intensity was lowered by a factor of five to decrease the extent of reaction. This experiment was conducted both to obtain a dose-response curve and to determine the magnitude of the mutagenic activity at an extent of reaction intermediate between those of the previous experiments. In Table 4.1-4 we present the steady-state reactant and product concentrations measured during the 20-h exposure (TA100 only, –S9). The indicated product distribution corresponds to that in Figure 4.1-1 near the beginning of the increase in O_3 concentration. In Figure 4.1-6 we present the dose-response curve obtained for this experiment. As witnessed by decreased survivor counts at 20 h, toxicity effects began to appear near 10 h. Because the change in toluene concentration, Δ toluene, for this experiment is only slightly larger than that for the previous τ = 6.7-h experiments, it appears that the response observed for this experiment is even larger than that observed at longer extent of reaction. It is possible that in the τ = 6.7-h exposures, toxicity effects resulted in smaller apparent mutagenic activities. It is clear, however, that the reaction of OH with toluene produces products that are significantly mutagenic, as determined by using the Ames test. The possible mutagenic products will be discussed in Section 5.

TABLE 4.1-4. REACTANT AND PRODUCT CONCENTRATIONS, PPB (TOLUENE/NO_X IRRADIATION, 8/86)

	Inlet	Effluent	% Yield (C/C)
Toluene	5145 (± 310)	4403 (± 144)	
NO	896	2	
NO_X	942	394	
O_3		153	
HNO_3		160	
CH_3COOH		47	1.8
PAN		85	3.3
HCHO		50	0.1
CH_3CHO		6	0.2
C_6H_5CHO		37	5.0
$(CHO)_2$		150	5.8
$CH_3C(O)CHO$		134	7.7

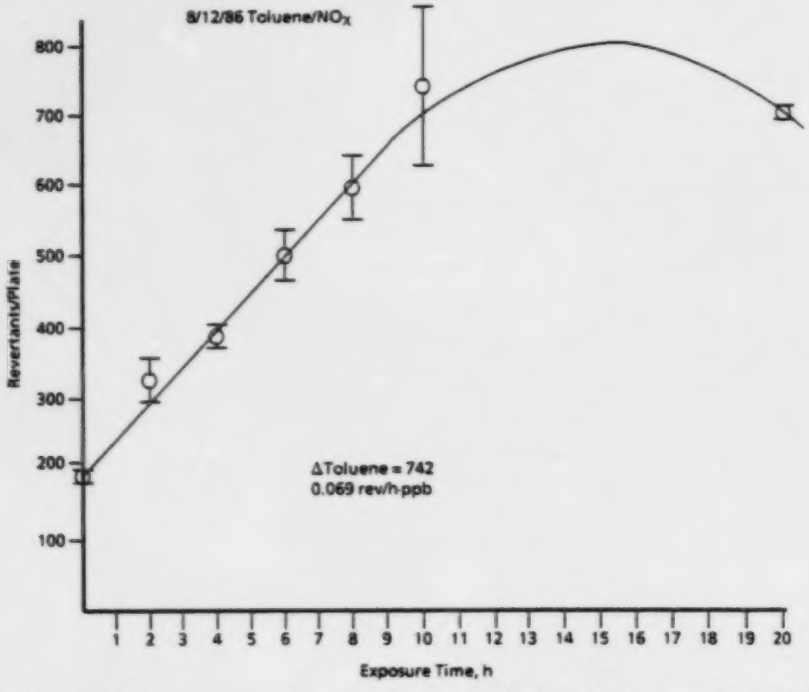

Figure 4.1-6. TA100 dose-response curve for toluene photooxidation products: 8/86, $\tau = 6.7$ h.

4.2 IRRADIATED PROPYLENE/NO_X MIXTURES

At the time of the initial toluene study, none of the identified products, including formaldehyde, benzaldehyde, glyoxal, and methylglyoxal, could account for the observed response (see Discussion). We were, at that point, faced with a difficult task in terms of accounting for the observed response. The photooxidation of toluene occurs through a very complex mechanism producing a large number of products with small yields, the majority of the products being unavailable commercially (46,47). The fact that the photooxidation of toluene led to mutagenic products was a very interesting result, but the identification of all the mutagenic products appeared to be a formidable task. We turned our attention to a photochemically simpler system and focused our efforts, once again, on determining the potential for the production of mutagenic products from the photooxidation of propylene. We present here the results of exposures of TA100 to the products of irradiated propylene/NO_X mixtures at both short and long extents of reaction. We also sought to isolate major subsystems of the photooxidation process and, therefore, examined the dark reactions of O_3 with propylene and of NO_3/N_2O_5 with propylene. For the C_3H_6/NO_X irradiations, the reaction chamber was operated at flows of 50 and 140 Lpm, yielding average residence times of 7.5 and 2.7 h, respectively. In the C_3H_6/N_2O_5 exposure, the average residence time was 2.7 h.

For the C_3H_6/NO_X irradiations the effluent from the reactor flowed through two exposure chambers in parallel. One chamber contained plates with TA100 and the other with TA100 plus S9. Surrogate plates for chemical analyses were placed in both chambers. The flow through the exposure chambers was 14 Lpm, yielding an exposure chamber residence time of 13.5 min. The exposures were conducted at 25°C for 20 h, and the total gas volume through each chamber was 17 m^3. Two additional exposure chambers were employed. Clean air was passed through one chamber and the reactant mixture through the second. Each chamber contained TA100, with and without metabolic activation, and two sets of surrogate plates.

For the C_3H_6/N_2O_5 exposure the two effluent exposure chambers contained 25 plates each of TA100 with and without S9. To one of these exposure chambers we added 0.7 ppm NO (continuously) to test for the presence of mutagenic peroxy nitrates (which are removed due to the presence of NO). For the C_3H_6/N_2O_5 exposure, a reactants exposure chamber was not employed since N_2O_5 would react with the water evaporated from the plates to produce gas-phase HNO_3, which is toxic to the bacteria.

Each of the exposures was conducted by adjusting the reactant concentrations to the desired levels in the inlet manifold and allowing the product distribution to reach a steady state in the chamber. The exposure chambers were then loaded with the covered test plates. The exposure

chambers were resealed, and the product concentrations in the exposure chambers were brought back to their steady-state levels. At this point the plates were uncovered, starting the exposures.

Figure 4.2-1 shows the time profiles of O_3, NO, NO_X–NO, C_3H_6, and PAN measured in the C_3H_6/NO_X static run, which had the same initial conditions as employed in the dynamic experiments. The NO_2 photolysis rate constant under these conditions was ~0.3 min^{-1}, and the dilution rate was 0.037 h^{-1}. The relative humidity was maintained at ~50%. In Figure 4.2-1, the vertical lines at 2.7 and 7.5 h indicate the product distributions predicted for dynamic experiments with residence times of 2.7 and 7.5 h, respectively, and performed under the same initial conditions as the static experiment. At 2.7 h, the reaction is still at a relatively early stage since there is approximately 20% (100 ppb) of the initial NO remaining. At 7.5 h, the reaction is near the O_3 and PAN maxima.

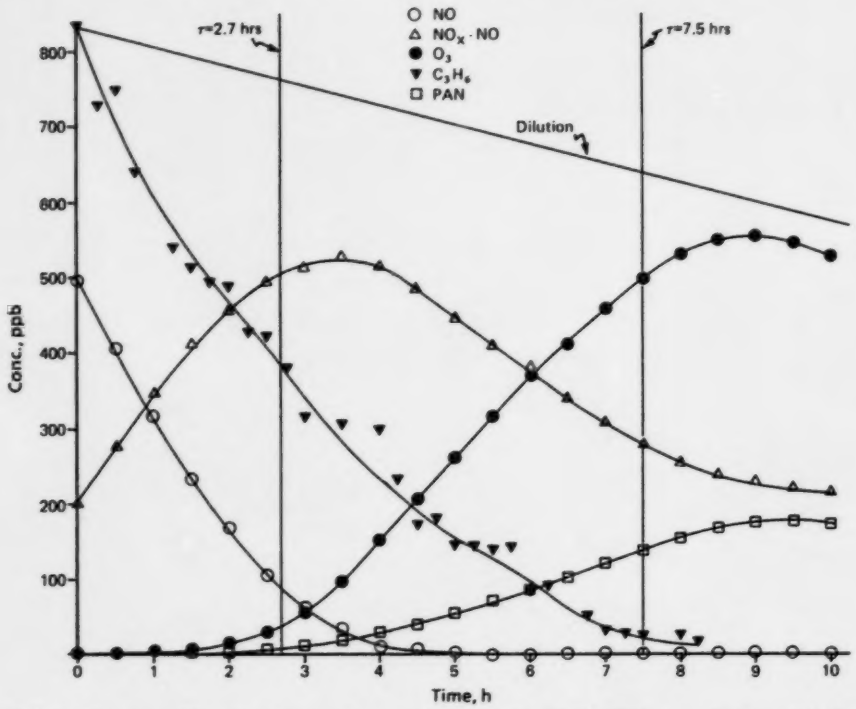

Figure 4.2-1. Time profiles for the major products in the C_3H_6/NO_X irradiation (static mode). The concentrations intersecting the vertical lines at 2.7 and 7.5 h represent the nominal product distributions for the two residence times.

The time profiles of HCHO, CH_3CHO, HNO_3, and HCOOH are given in Figure 4.2-2. The aldehydic concentration difference between the two residence times is minimal. Nitrate extracted from nylon filters is generally identified with HNO_3. In this experiment, the measured $HCOO^-$ is associated with HCOOH. For conditions under which the sampling was performed, the detection limit for HCOOH is 15 ppb. Figure 4.2-3 shows the formation of three organic nitrates produced

during the photooxidation: propylene glycol dinitrate (PGDN), 2–hydroxypropyl nitrate (2–HPN), and 2–nitratopropyl alcohol (2–NPA). The reaction of OH with C_3H_6 in the presence of O_2 and NO forms 2–HPN and 2–NPA as stable products.

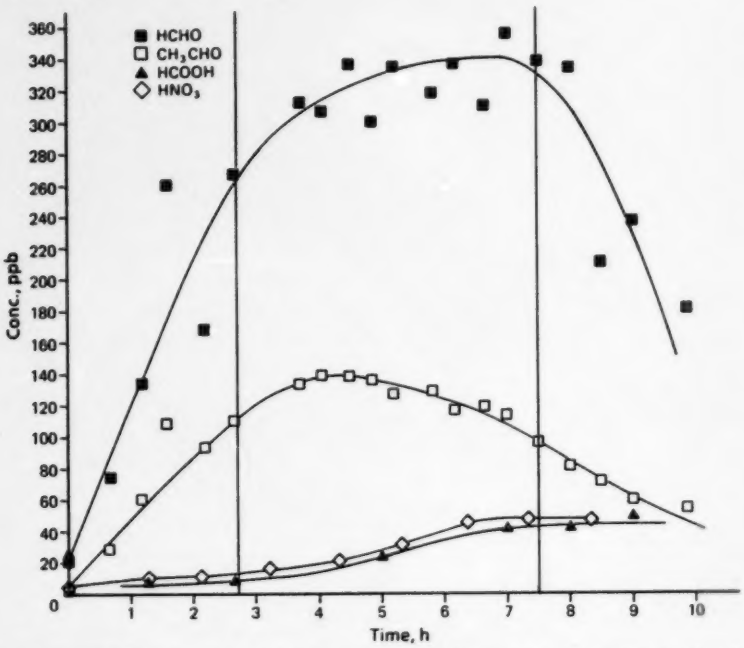

Figure 4.2-2. Time profiles for the aldehydes and nitric and formic acids in the C_3H_6/NO_X irradiation.

The dynamic experiments had the same initial concentrations as the static experiment, within experimental uncertainty. The inlet concentrations, total flow, and irradiation intensity remained constant throughout the dynamic experiments and yielded steady-state product distributions. The average values for the observed product concentrations in the reaction chamber for each of the two dynamic experiments are given in Table 4.2-1. These concentrations can be compared with those of the static experiment by examining Figures 4.2-1, 4.2-2, and 4.2-3 at the appropriate extent of reaction. A nominal correspondence between the two experiments can be seen.

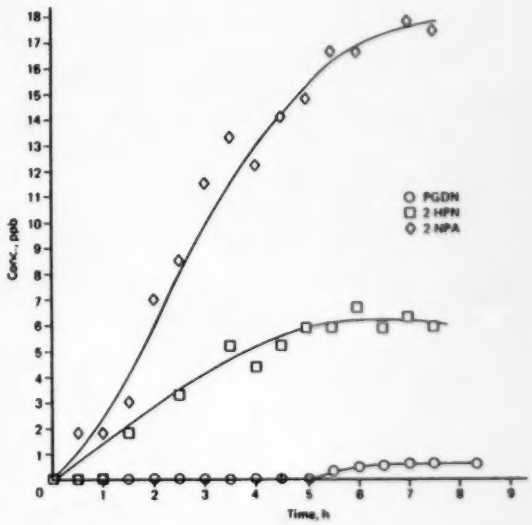

Figure 4.2-3. Time profiles for nitrates in the C_3H_6/NO_X irradiation.

TABLE 4.2-1. AVERAGE REACTANT AND PRODUCT CONCENTRATIONS (ppb) FOR C_3H_6/NO_X IRRADIATION (DYNAMIC MODE)

Compound	Input Concentration	Effluent Concentration (τ = 2.7 h)	Effluent Concentration (τ = 7.5 h)
C_3H_6	826 ± 33	548 ± 24	100 ± 13
NO	505 ± 24	155 ± 29	BD†
$NO_X - NO$	200 ± 39	521 ± 38	347 ± 21
O_3	–	18 ± 7	451 ± 29
HCHO	–	219 ± 32	247 ± 71
CH_3CHO	–	118 ± 8	90 ± 4
PAN	–	6 ± 3	181 ± 12
HNO_3	–	40 ± 10	73 ± 17
CH_3ONO_2	–	1.2 ± 0.2	1.4 ± 0.2
PGDN	–	BDL	0.8 ± 0.2
2-HPN	–	1.8 ± 0.4	3.0 ± 1.1
2-NPA	–	5.6 ± 1.1	10.4 ± 2.6
CO	81	163	–
RH* (%)	–	52	65
HCOOH	–	BDL	40

* RH - relative humidity in units of percent.
† BDL - below detection limit.

The gas-phase products in the exposure chambers were measured before and during the exposure of the biological assay. These values are used as one means of estimating the amount of material deposited into each plate. The net deposition of material into the plates during the exposure is tabulated in Table 4.2-2 under the heading "Chamber Decrease." These values are calculated from the relationship given in Expression II:

$$X_i\left(\frac{\mu mol}{plate}\right) = \left(\frac{P_i(R) - P_i(E)}{RT}\right)\left(\frac{V_E}{N_P}\right) \qquad \text{(II)}$$

where X_i is the plate concentration of species i; $P_i(R)$ and $P_i(E)$ are the partial pressures (μatm) of species i in the reaction and exposure chambers, respectively; V_E is the volume of effluent passing through the exposure chamber; and N_p is the total number of plates. The amounts of material presented in Table 4.2-2 represent upper limits since some of the material may also be lost to the exposure chamber walls.

TABLE 4.2-2. C_3H_6/NO_X IRRADIATIONS: CONCENTRATIONS OF SPECIES DETECTED FROM EXPOSURE CHAMBER DECREASE (Exp. II) AND/OR APPEARANCE IN THE SURROGATE PLATES*

	τ = 2.7 h		τ = 7.5 h	
Compound	Chamber Decrease	Surrogate Plates	Chamber Decrease	Surrogate Plates
HCHO	1.62	1.15	2.28	1.65
PAN	0.048	–	0.89	1.48‡
HNO_3	–†	0.085	0.78	0.83
CH_3ONO_2	0.001	–	0.004	–
PGDN	0	–	0.0024	–
2-HPN	0.017	–	0.034	–
2-NPA	0.047	–	0.10	–
$HCOO^-$	–	0.21	–	1.29

* Concentration given as μmol/plate.
† Not measured.
‡ From nitrite measurement.

A second method for determining the amount of material deposited into the biological assay is from the analysis of the surrogate plates. The results for each measured compound are given in Table 4.2-2. A comparison of the results for the two methods can be made for HCHO, PAN, and HNO_3. In each case, the agreement is within 50%. This enhances the confidence for a species that can be measured using only one of the techniques.

The mutagenic activities observed for the C_3H_6/NO_X photooxidation mixture are presented in Table 4.2-3. Three sets of experiments are presented, each with its own set of laboratory controls and background measurements. The first three rows in Table 4.2-3 represent laboratory measurements of the characteristics of the particular assay used in the experiment. The "spontaneous" plates measure the natural reversion rate observed under sterile conditions in the laboratory. These serve as a check to establish the viability of the bacteria. The strain sensitivity is

determined by adding 1.0 and 0.5 μg, respectively, of known mutagens, sodium azide and 2-aminoanthracene, to TA100 and TA100 + S9, respectively. A clean air control chamber was employed to measure the background reversion rate in the ambient environment under which the photochemical experiments take place. In addition, a reactants exposure chamber, which samples the reactant gases directly from the inlet manifold, was used to check for mutagenic activity of the reactants. The clean air irradiation (Table 4.2-3) is a separate experiment that serves as a check to ensure that the observed revertant level is due to the photochemical effluent rather than a chamber artifact. The revertant levels at both residence times show increased values over the clean air control. However, the mutagenic activity for the long residence time is substantially greater than that for the short residence time. The addition of S9 metabolic activation does not statistically increase the mutagenic activity, relative to those plates without S9 mix, indicating that the mutagens present are direct acting.

TABLE 4.2-3. MEASURED MUTAGENIC ACTIVITY FOR EXPOSURE OF TA100 TO THE EXPERIMENTAL GAS STREAMS IN REVERTANTS/PLATE (± 1σ) FOR THE C_3H_6/NO_X IRRADIATIONS

Exposure Condition	Clean Air Irradiation		τ = 2.7 h		τ = 7.5 h	
	w/o S9	w/S9	w/o S9	w/S9	w/o S9	w/S9
Spontaneous	164 ± 12	178 ± 11	142 ± 10	131 ± 8	184 ± 29	131 ± 18
15.4 nmol sodium azide	909 ± 73	–	919	–	1388	–
2.6 nmol 2-amino-anthracene	–	1525	–	421	–	536
Clean air chamber*	361 ± 60	379 ± 70	194 ± 15	225 ± 32	278 ± 92	290 ± 58
Reactants chamber*	356 ± 48	363 ± 67	261 ± 41	293 ± 54	294 ± 58	268 ± 44
Effluent chamber†	374 ± 59	404 ± 69	356 ± 69	373 ± 48	903 ± 69	968 ± 101

* Numbers are averages for ~25 plates.
† Numbers are averages for ~50 plates.

The τ = 2.7-h and τ = 7.5-h exposures were repeated in 1/86 (roughly 1.5 years later) in experiments in which groups of plates were covered throughout the exposures. The product distributions were nearly identical to those previously observed. The dose-response curves obtained for the τ = 2.7-h and τ = 7.5-h experiments are presented in Figures 4.2-4 and 4.2-5, respectively. In both cases it can be seen that the initial part of the curve is reasonably linear, but that toxic effects begin to occur near 10 h. For the τ = 7.5-h exposure the survivor counts were substantially lower

beyond a 10-h exposure time. In agreement with the earlier experiments, the response at the $\tau = 7.5$-h residence time was at least twice as large as that observed for the $\tau = 2.7$-h residence time. It is clear from these experiments that mutagenic products are produced from the photooxidation of propylene.

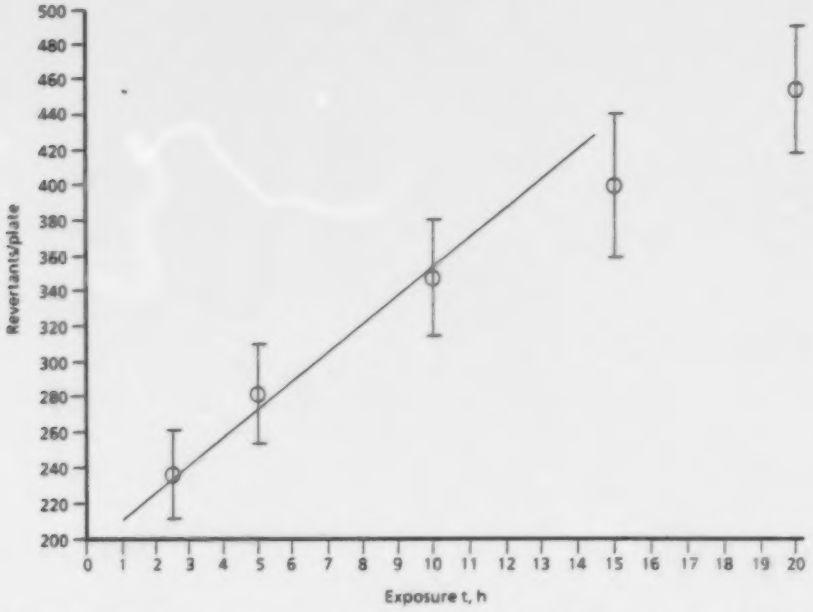

Figure 4.2-4. Dose-response curve for $\tau = 2.7$-h irradiated C_3H_6/NO_X mixture, TA100.

To determine the extent to which NO_3 reaction with C_3H_6 may lead to mutagenic products that could account for the large mutagenic activity observed in the irradiated C_3H_6/NO_X system (at $\tau = 7.5$ h), we conducted a C_3H_6/N_2O_5 exposure.

As mentioned previously, to one effluent stream we added NO at ~ 0.7 ppm. It has been reported (48) that the reaction of C_3H_6 with NO_3 leads to the production of large yields of nitroxyperoxypropyl nitrate (NPPN). Since we have shown that PAN (a peroxy nitrate) is a mutagen with TA100 (see Section 4.6), it is possible that NPPN or

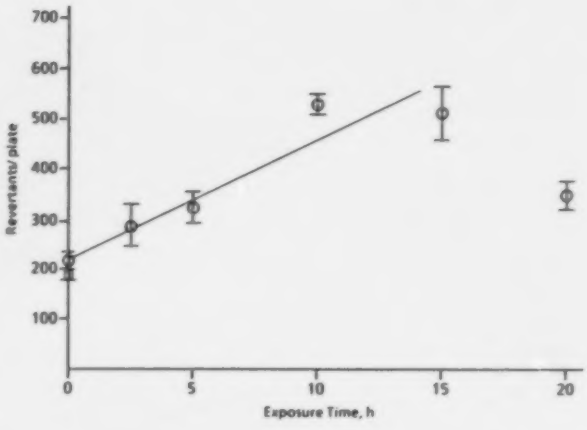

Figure 4.2-5. Dose-response curve for $\tau = 7.5$-h irradiated C_3H_6/NO_X mixture, TA100.

other peroxy nitrates formed may be mutagenic as well. Peroxy nitrates such as PAN are in equilibrium with their respective peroxy radical and NO_2 (49), so they can be removed via NO addition as shown in Reactions 11 and 12 below.

$$RO_2NO_2 \rightleftharpoons RO_2+NO_2 \qquad 11,-11$$

$$RO_2+NO \rightarrow RO+NO_2 \qquad 12$$

where R is an organic group. The resultant alkoxy radical would then be removed by reaction with O_2 or NO_2 or by unimolecular decomposition. The presence of mutagenic peroxy nitrates can therefore be checked by comparison of the results for the two exposure chambers, since these compounds will not be present in the exposure chamber with added NO.

The average inlet and effluent reactant concentrations, chamber parameters, and steady-state concentrations for the products measured in the C_3H_6/N_2O_5 exposure are presented in Table 4.2-4. This experiment was conducted without added humidity.

TABLE 4.2-4. AVERAGE REACTANT AND PRODUCT STEADY-STATE CONCENTRATIONS FOR THE C_3H_6/N_2O_5 EXPOSURE

Parameter Measured*	Inlet	Effluent
Inlet Reactant Flow, L/min	140 ± 0.085	
NO_X	1.76 ± 0.20	1.94 ± 0.19
C_3H_6	1.26 ± 0.04	1.00 ± 0.04
HCHO		0.017 ± 0.004
CH_3CHO		0.023 ± 0.007
α-nitratoacetone		0.032 ± 0.011
PGDN		0.002 ± 0.001
2-HPN		0.008 ± 0.002
2-NPA		0.002 ± 0.001
PAN		0.020

* Concentrations in parts per million.

In this experiment, C_3H_6 and N_2O_5 were diluted and mixed together in the inlet manifold. It was possible that they could react as shown in Reactions -2 and 13,

$$NO_3 + C_3H_6 \rightarrow Products \qquad 13$$

prior to entering the reaction chamber. In a separate C_3H_6/N_2O_5 static experiment under similar conditions (44), we observed a value for $\Delta N_2O_5/\Delta C_3H_6$ of roughly 2:1. Since ΔC_3H_6 in this experiment is 0.26 ppm, the assumption can be made that the initial N_2O_5 concentration was ~0.5 ppm. This then leads to an initial NO_2 concentration of ~0.8 ppm, given the total NO_X value of 1.8 ppm listed in Table 4.2-4. By use of values for k_2 and k_{-2} of 4.6×10^{-12} cm^3 $molecule^{-1}s^{-1}$ and $3.7 \times 10^{-1}s^{-1}$, respectively (50), an equilibrium NO_3 concentration of 2.2 ppb is estimated. Using the value $k_{13} = 4.2 \times 10^{-15}$ cm^3 $molecule^{-1}s^{-1}$ (51), and a residence time in the inlet manifold of 1 min, we calculate that $\leq 1\%$ C_3H_6 reacted in this time. Therefore, the inlet manifold concentrations can be taken as the starting concentrations.

The mechanisms for formation of the products listed in Table 4.2-1 have been presented elsewhere (44,48). A mass balance of the product concentrations from Table 4.2-4 indicates that we cannot account for a large part of the reacted C_3H_6. It may be that much of it is present as NPPN, which we could not detect. The PAN concentration was measured as 0.020 ppm in a separate experiment under equivalent conditions.

The results of the bioassays performed are presented in Table 4.2-5, along with the laboratory controls. The final plate concentrations for each product of the C_3H_6/N_2O_5 reaction are presented in Table 4.2-6, as calculated from Expression II.

As shown in Table 4.2-5, the two sets of effluent chamber plates exhibit induced revertant levels of approximately 180 revertants/plate above the clean air values. There seems to be no significant difference between those with and without metabolic activation, indicating that the mutagens present are direct acting. In addition, no significant difference was observed in the results for the two effluent exposure chambers, one of which had NO added. Although this may indicate that the peroxy nitrates present are not mutagenic, their transfer efficiencies to the exposure chambers may be low, or they may be deposited on the reaction chamber walls during the 2.7-h average residence time. Bandow *et al.* (48) observed a significant wall loss rate for NPPN in their reaction chamber.

TABLE 4.2-5. OBSERVED MUTAGENIC ACTIVITIES IN REVERTANTS PER PLATE FOR THE C_3H_6/N_2O_5 EXPOSURE

Exposure Condition	TA100	TA100 + S9
Spontaneous	133 ± 13	122 ± 21
15.4 nmol sodium azide	668	
2.6 nmol 2-aminoanthracene		590
clean air chamber*	180 ± 31	169 ± 33
effluent chamber*	375 ± 122	351 ± 102
effluent (+ NO) chamber*	323 ± 81	369 ± 112

* Numbers are averages for ~25 plates.

TABLE 4.2-6. CONCENTRATIONS OF PRODUCTS (C_3H_6/N_2O_5 EXPOSURE) DETECTED IN MICROMOLES PER PLATE AS CALCULATED FROM EXP. II

Product	µmol/plate (max. - Exp. II)
HCHO	0.2
HNO_3	<0.6
PAN	0.2
2-HPN	0.1
2-NPA	0.02
PGDN	0.02
CH_3ONO_2	--
HCOOH	--
$CH_3C(O)CH_2ONO_2$	0.4

In this experiment, we showed that chemical mutagens are produced from the reaction of NO_3 with C_3H_6. However, a computer modeling study we conducted of the C_3H_6/NO_X irradiated system indicated that NO_3 reaction with C_3H_6 would have occurred only to the extent of ~0.010 ppm at 7.5 h. Since ΔC_3H_6 due to NO_3 reaction in the C_3H_6/N_2O_5 exposure was 0.26 ppm, we can estimate that

NO_3 reactions would have contributed on the order of only 10 revertants/plate in the irradiated C_3H_6/NO_X system at 7.5 h.

Because the mutagenic activity at long residence time, when O_3 was present, was substantially greater than at short residence time, it seemed reasonable to determine whether the products of ozone reaction with propylene may account for the observed response in the irradiated C_3H_6/NO_X system. We conducted exposures of TA100 to the products from the reaction of 5.4 ppm C_3H_6 with 0.90 ppm O_3 in the dark at $\tau = 2.7$ h. In addition, an experiment was conducted in which 0.21 ppm NO_2 was also added to this mixture to test for the possibility that radicals produced in the ozonolysis might react with NO_2 to produce mutagenic organic nitrates. For both experiments, ΔC_3H_6 was ~1.0 ppm and the overwhelmingly predominant products were acetaldehyde and formaldehyde. The observed mutagenic activities for these two exposures are presented in Figure 4.2-6.

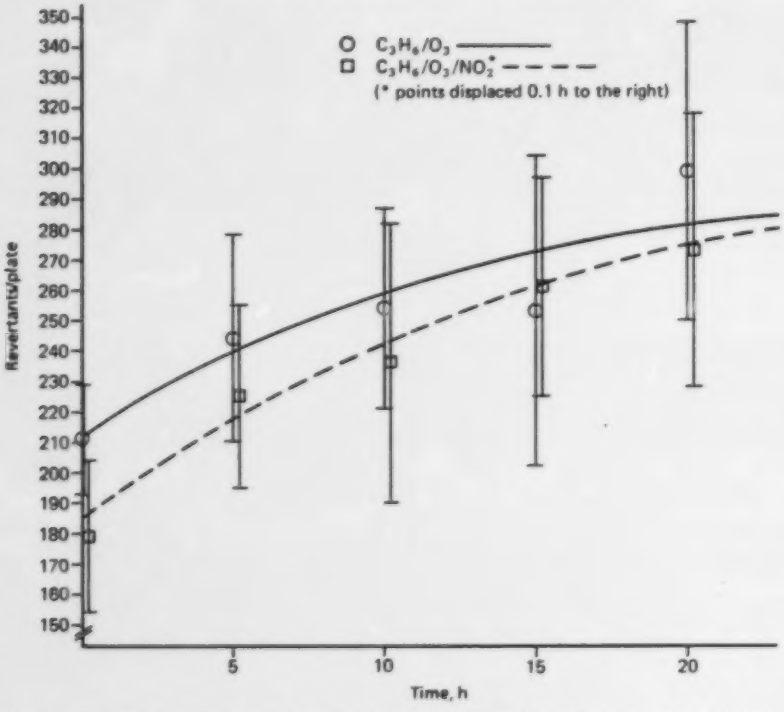

Figure 4.2-6. Dose-response curve for products of propylene ozonolysis, TA100.

As shown in Figure 4.2-6, the number of revertants per plate increases measurably (but nonlinearly) with exposure time. The survivor data (not presented here) indicate that toxicity effects may become important for exposures of 10 h or more. It is clear from these experiments that mutagenic organic nitrates are not formed as a result of addition of NO_2. The principal objective of

these experiments was to attempt to determine the extent to which C_3H_6 reactions with O_3 could account for the large response (~600 excess revertants/plate) observed in the irradiated C_3H_6/NO_X system. Computer simulations of the irradiated C_3H_6/NO_X system, under the conditions of that exposure, indicated that only ~0.20 ppm of the reacted C_3H_6 had been removed by reaction with ozone. Because the ozone plus propylene dark experiment (Figure 4.2-6) involved five times as much reaction, it appears that the products of the C_3H_6/O_3 reaction could not have caused a significant response in the C_3H_6/NO_X irradiations. It will be shown in the Discussion section that formaldehyde could account for the entire response observed in these C_3H_6/O_3 experiments.

An exposure of TA100 to the products of an irradiated ethylene/NO_X mixture ($\tau = 2.7$ h) was conducted to determine whether HCHO and the inorganic photooxidation products present might yield a significant mutagenic activity. For this experiment the inlet C_2H_4 and NO_X concentrations were 1.16 and 0.28 ppm, respectively, and ΔC_2H_4 was 0.54 ppm. The steady-state O_3 and HCHO concentrations were 0.47 and 0.33 ppm, respectively. In addition, concentrations of HNO_3, H_2O_2, and HO_2NO_2 should have been comparable to those present in the long residence time C_3H_6/NO_X experiments. The results of the ~20-h exposure of TA100 to this mixture indicated only ~35 excess revertants/plate (relative to the clean air and reactant controls). This response is essentially what would be expected based on the mutagenic activity of formaldehyde (see Discussion). It is clear from this experiment that the inorganic photooxidation products did not contribute significantly to the observed mutagenic activities in the C_3H_6/NO_X (and toluene/NO_X) experiments.

4.3 IRRADIATED CH_3CHO/NO_X MIXTURES

Our initial attempts at accounting for all the mutagenicity in the C_3H_6/NO_X system indicated that the two major products found to be mutagenic, PAN and HCHO (see Discussion), were not mutagenic enough to account for a significant fraction of the total response. Because the response was much larger at longer residence time, we began to consider whether secondary reactions involving acetaldehyde could be responsible for the production of the mutagenic products. To investigate this possibility, we conducted a series of experiments with acetaldehyde similar to those conducted with propylene (i.e., 20-h exposures of TA100 to the photooxidation products of acetaldehyde under conditions of both short [some NO present] and long [near the ozone maximum] extents of reaction).

To determine the temporal behavior of the reactants and products in the irradiated CH_3CHO/NO_X system, we conducted a static-mode irradiation. For this experiment the reaction chamber operated as a conventional smog chamber; that is, the reactants were added through the mixing manifold to the desired initial concentrations, the lights were turned on, and dilution air was added at 10 L/min to account for an equivalent sampling rate at the effluent end of the chamber. The initial reactant concentrations were 1440, 418, and 436 ppb for CH_3CHO, NO, and NO_X, respectively. Figure 4.3-1 shows the time profiles for CH_3CHO, PAN, HCHO, CH_3ONO_2, NO_X-NO (which is associated with PAN + NO_2), NO, and O_3.

We present here the results of two dynamic-mode exposures under conditions in which the product distributions should have been considerably different. One was conducted with input concentrations of 1237, 406, and 427 ppb of CH_3CHO, NO, and NO_X, respectively, and a residence time of 2.6 h. The product distribution was similar to that at ~1.5 h in Figure 4.3-1. Under these conditions, where considerable NO is present, the product distribution is simplified because peroxy radical-radical reactions will not occur (see Reactions 14-24). The other exposure was conducted at inlet concentrations of 1190, 31, and 212 ppb of CH_3CHO, NO, and NO_X, respectively. The product distribution at steady state was similar to that at ~6 h in Figure 4.3-1. (Only NO_X is substantially higher in the static experiment at 6 h. NO_2 is the major component of difference since the PAN concentrations are similar for the static and dynamic [$\tau = 4.0$ h] experiments.) Under these conditions, organic peroxides can be produced and the product mixture is considerably more complex. The average chamber inlet and effluent reactant and product concentrations for these two exposures are presented in Table 4.3-1.

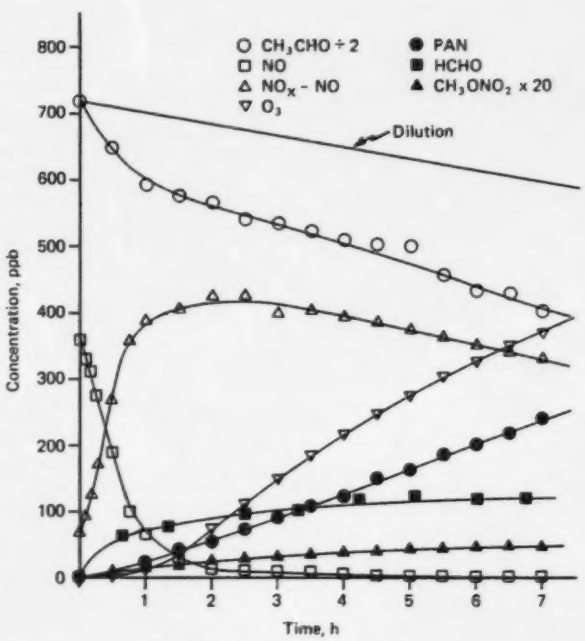

Figure 4.3-1. $CH_3CHO/NO/NO_2$ static-mode irradiation.

TABLE 4.3-1. AVERAGE REACTANT AND PRODUCT CONCENTRATIONS (ppb) FOR IRRADIATED CH_3CHO/NO_X EXPOSURES

	$\tau = 2.6$ h		$\tau = 4.0$ h	
Compound	Input Conc.	Effluent Conc.	Input Conc.	Effluent Conc.
CH_3CHO	1237 ± 124	1064 ± 113	1190 ± 26	977 ± 58
NO_X	427 ± 26	388 ± 22	212 ± 13	174 ± 10
NO	406 ± 35	40 ± 4	31 ± 3	5 ± 2
O_3	–	40 ± 7	–	311 ± 12
PAN	–	102 ± 7	–	171 ± 11
HCHO	–	72 ± 6	–	101 ± 16

The results of both exposures are presented as dose-response curves in Figure 4.3-2. For these experiments the numbers at each exposure period are averages for ~25 plates. Throughout the $\tau = 2.6$-h exposure there was no discernible change in the survivor levels. For the $\tau = 4.0$-h exposure the survivor counts were 675, 742, 552, and 0, for 2.5, 5.0, 10.0, and 20.0 h, respectively, indicating the potential for toxicity effects in the latter part of the exposure. Although there appears to be some curvature for both dose-response curves, we have drawn straight lines representing the initial slopes to facilitate comparison of the mutagenic activities of the two mixtures. As can be seen in Figure 4.3-2, for a 20-h exposure at long extent of reaction ($\tau = 4.0$ h) we observed roughly 400 excess revertants/plate, a clearly significant response. In fact, given the amount of acetaldehyde consumed under these conditions (210 ppb), and given our estimate of the amount of acetaldehyde that had reacted under the conditions of the long residence time propylene experiments, it is clear that the photooxidation products of acetaldehyde can account for essentially all of the mutagenic activity associated with the propylene system (at long extent of reaction).

Given the important role propylene and acetaldehyde play in urban photochemistry, there is a strong motivation to attempt to identify the species that caused the observed mutagenic activity. It is important to recognize that there was also a significant response at the short extent of reaction, under conditions in which NO was present. In fact, when the responses were normalized to the amount of CH_3CHO consumed, the mutagenic activities of the two mixtures were essentially the

same. An important point in this regard is that the mechanism for the photooxidation of CH_3CHO is very simple under conditions in which NO is present (52).

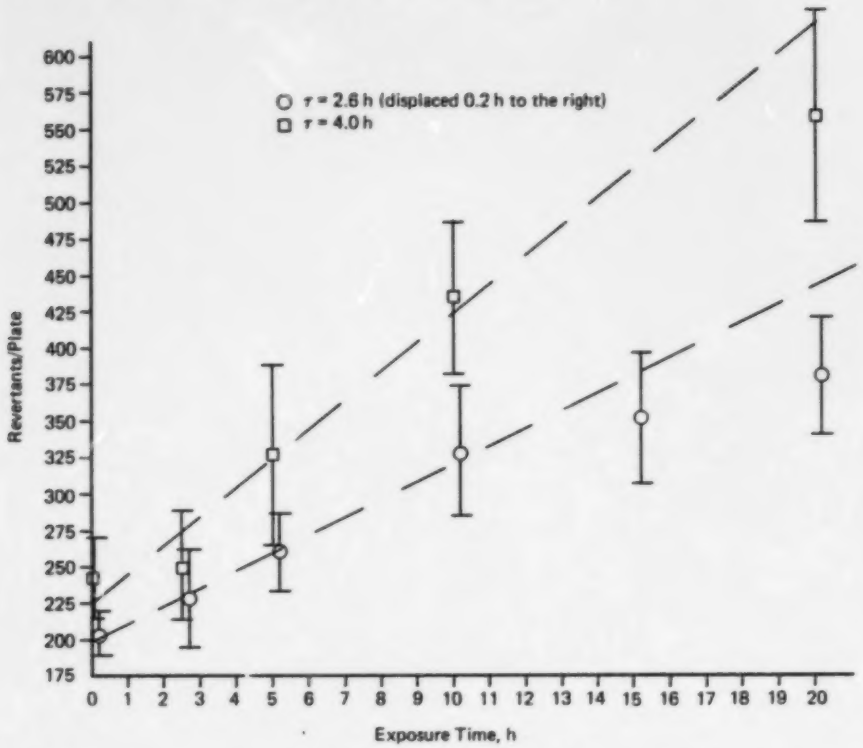

Figure 4.3-2. Dose-response curves for irradiated CH_3CHO/NO_X exposures.

Under these conditions, the reaction proceeds as shown in Reactions 14-24 below.

$$CH_3CHO + OH \xrightarrow{O_2} CH_3C(O)O_2 + H_2O \qquad 14$$

$$CH_3C(O)O_2 + NO_2 \rightleftharpoons CH_3C(O)O_2NO_2\,(PAN) \qquad 15,\text{-}15$$

$$CH_3C(O)O_2 + NO \rightarrow CH_3C(O)O + NO_2 \qquad 16$$

$$CH_3C(O)O \xrightarrow{O_2} CH_3O_2 + CO_2 \qquad 17$$

$$CH_3CHO + h\nu \xrightarrow{O_2} CH_3O_2 + CO + HO_2 \quad (18)$$

$$CH_3O_2 + NO \rightarrow CH_3O + NO_2 \quad (19)$$

$$CH_3O + O_2 \rightarrow HCHO + HO_2 \quad (20)$$

$$CH_3O + NO_2 \rightarrow CH_3ONO_2 \quad (21)$$

$$CH_3O + NO \rightarrow CH_3ONO \quad (22)$$

$$CH_3ONO + h\nu \rightarrow CH_3O + NO \quad (23)$$

$$HO_2 + NO \rightarrow OH + NO_2 \quad (24)$$

Because the photolysis of methyl nitrite (CH_3ONO) is fast (52), it is unlikely that the concentration of CH_3ONO reaches significant levels. Thus, the only organic products measured in the presence of NO are PAN, HCHO, and much smaller amounts of methyl nitrate (CH_3ONO_2). One of these three species must account for the majority of the mutagenic activity in the CH_3CHO/NO_X experiments, and, therefore, as indicated above, in the C_3H_6/NO_X irradiation as well. As discussed in Section 4.6 and in the Discussion section of this report, that species is PAN.

4.4 IRRADIATED ALLYL CHLORIDE/NO_X MIXTURES

To determine whether chlorinated hydrocarbons might yield more mutagenic products than their nonchlorinated analogues, we conducted a series of static-mode irradiations and exposures in which we attempted to measure the mutagenic activity of the products of the photooxidation of allyl chloride. We report here the results of four static-mode smog chamber irradiations (Experiments A-D) of allyl chloride (C_3H_5Cl)/NO_X and $C_3H_5Cl/C_2H_6/NO_X$ mixtures in which TA100 was periodically exposed to the chamber effluent during the reaction profiles. Because the photooxidation of allyl chloride proceeds through OH-radical and Cl-atom chain reactions (32), the experiments with ethane were conducted to allow measurement of the mutagenic activity of the allyl chloride photooxidation products in the presence and absence of Cl-atom reaction products

(C_2H_6 is used here as a Cl-atom scavenger). Because of the magnitude of the observed mutagenic activity it was possible to conduct these experiments with the chamber operated in a static mode (i.e., as a conventional smog chamber). This enabled us to determine mutagenicity profiles as the reaction proceeded.

For the four experiments conducted, the initial NO and NO_2 concentrations were ~350 and 115 ppb, respectively. In the first two experiments (A and B), the initial allyl chloride concentrations were ~725 ppb. In Experiment B we also added ~11 ppm C_2H_6, which had no significant effect on the overall reaction rate. The light intensity was identical for Experiments A and B (we estimate the rate constant for NO_2 photolysis to be ~0.2 min^{-1}). For Experiments C and D the initial ethane concentration was ~150 ppm, and the overall reaction rate was therefore dominated by OH reaction with ethane. Experiment C contained no allyl chloride, and in Experiment D the initial allyl chloride concentration was 850 ppb. The light intensity was identical for Experiments C and D, but was 0.4 times that used for Experiments A and B (we estimate the NO_2 photolysis rate constant to be ~0.08 min^{-1}). From the results of these experiments we attempted to determine the mutagenic activity of the products of OH (and O_3) reaction with allyl chloride as compared with the mutagenic activity of the Cl-atom reaction products.

The results for Experiments A through D are presented in Figures 4.4-1 – 4.4-4, respectively, which include concentration data for the reactants and some of the major products (e.g., PAN) for each exposure. The Ames test data (± 1 S.D.) are also included in these plots for the effluent exposure chamber as revertants/plate per 15-min exposure (Figures 4.4-1 and 4.4-2), or revertants/plate per 30-min exposure (Figures 4.4-3 and 4.4-4). The clean air exposures (which lasted the duration of the irradiations) yielded 180 ± 21, 212 ± 14, 220 ± 10, and 194 ± 17 revertants/plate for Experiments A-D, respectively. For Experiment A (irradiated allyl chloride/NO_x), the first three exposure periods lasted 15 min. However, experiments we performed previously under essentially identical reaction conditions indicated that 15-min exposures at longer extents of reaction (i.e., near the ozone maximum) led to toxicity effects (as witnessed by decreased survivor levels). Therefore, for Experiments A and B, the last three exposure periods were 5 min in duration. For Figures 4.4-1 and 4.4-2 the bioassay data are all normalized to a 15-min exposure period. This was done for the 5-min exposures by subtracting the control (covered) plate revertant counts from the observed test plate counts, multiplying the result by three, and then adding to this value the control plate counts. Although this correction assumes a linear dose-response curve, for all exposures the survivor plates indicated no evidence of toxicity. As shown in Figure 4.4-1, the number of revertants/plate for the allyl chloride/NO_x irradiation increased to ~1700 (nearly 10 times the control level) for 15-min exposures, at a reaction time of 4.5 h. We note that, from the initial exposure period, allyl chloride alone does not yield a response even though it has been reported to be a weak mutagen (53). This is

probably due to the fact that allyl chloride, being volatile and relatively nonpolar, does not deposit into the plates during the exposure. Although it appears from Figure 4.4-1 that the mutagenic activity increases as the extent of reaction increases, the mutagenic activity normalized to the amount of allyl chloride consumed is nearly constant throughout the reaction. In fact, near the end of the reaction, the mutagenic activity decreased, probably because Cl atoms began reacting with the products rather than with allyl chloride (see Discussion).

In a previous study of the kinetics and mechanism of the photooxidation of allyl chloride (32), we found that a significant fraction of the allyl chloride removal was due to reaction with Cl atoms that are produced from OH reaction with allyl chloride. It is possible, therefore, that the results of Experiment A are not representative of the atmospheric photooxidation products of allyl chloride, since under atmospheric conditions, we would expect very little reaction of the Cl atom with allyl chloride. To test for this possibility, we conducted Experiment B with ethane added as a Cl-atom scavenger. Ethane represents a good Cl-atom scavenger for this study because its Cl-atom reaction rate constant (at 298K) is large, 6.38×10^{-11} $cm^3 \cdot molecule^{-1} \cdot s^{-1}$ (54) and its OH radical rate constant is much smaller, 2.74×10^{-13} $cm^3 \cdot molecule^{-1} \cdot s^{-1}$ (55). We reported a rate constant of 1.7×10^{-11} $cm^3 \cdot molecule^{-1} \cdot s^{-1}$ for OH reaction with allyl chloride at 298K (32). To estimate the relative rate of Cl-atom reaction with allyl chloride vs. ethane, we recently measured the rate constant for Cl-atom reaction with allyl chloride by the relative rate method described by Atkinson and Aschmann (54), using ethane as the reference compound. Using the chromatographic procedures described in the Experimental section of this report we found $k_{(Cl + C_3H_5Cl)} = 2.3 (\pm 0.1) \times 10^{-10}$ $cm^3 \cdot molecule^{-1} \cdot s^{-1}$, using $k_{(Cl + C_2H_6)} = 6.38 \times 10^{-11}$ $cm^3 \cdot molecule^{-1} \cdot s^{-1}$. Therefore, under the conditions of Experiment B (720 ppb allyl chloride, 11.1 ppm ethane) the initial rate of OH reaction with allyl chloride was 4.0 times faster than the rate of OH reaction with ethane. Under these conditions, then, the presence of 11.1 ppm ethane should have only a small impact on the overall reaction rate (e.g., conversion of NO to NO_2). As can be seen by comparing the data in Figures 4.4-1 and 4.4-2, the time of the NO_2 maximum increased from 2 to 2.5 h, respectively. However, the rate of Cl-atom reaction with ethane is 4.3 times faster than with allyl chloride. We therefore estimate that the yield of products due to Cl-atom addition to allyl chloride would be roughly five times smaller in Experiment B relative to Experiment A. From inspection of Figure 4.4-2, it is clear that the number of revertants per plate in Experiment B is significantly smaller than in Experiment A. In fact, the number of excess revertants (i.e., relative to the zero reaction time exposure) at 4.5 h (~230 revertants/plate) is six to seven times smaller than that observed in the absence of ethane. When normalized to the amount of allyl chloride consumed, the mutagenic activity of the products is three to four times smaller. This result suggests that the majority of the response observed in the allyl chloride/NO_x irradiation (Experiment A) was due to the presence of Cl-atom addition products.

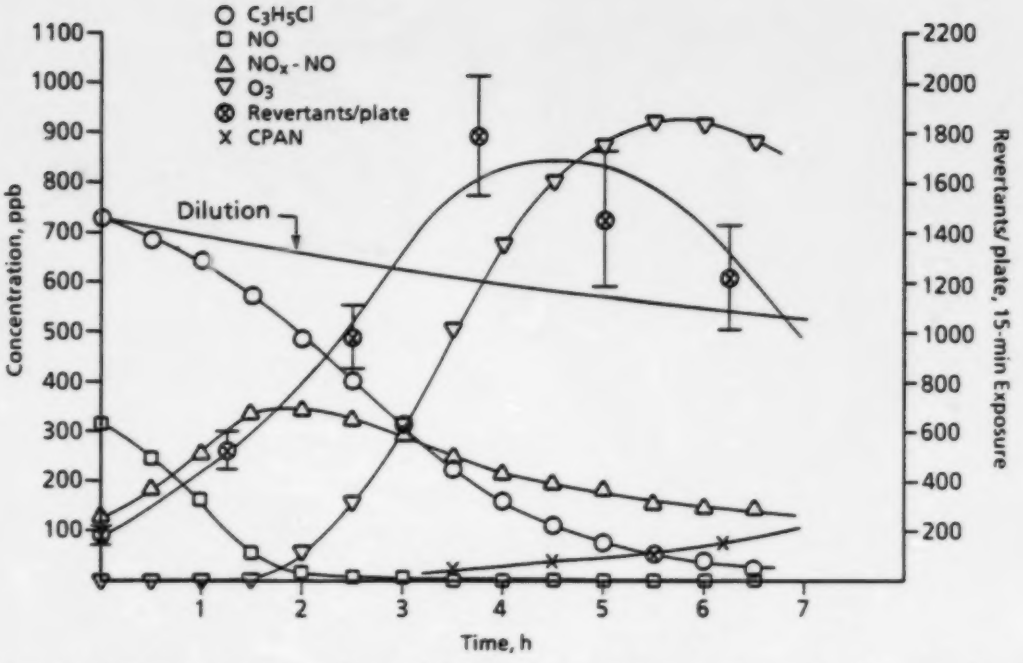

Figure 4.4-1. Experiment A: reaction chamber component concentrations and mixture mutagenic activity, C_3H_5Cl/NO_X irradiation.

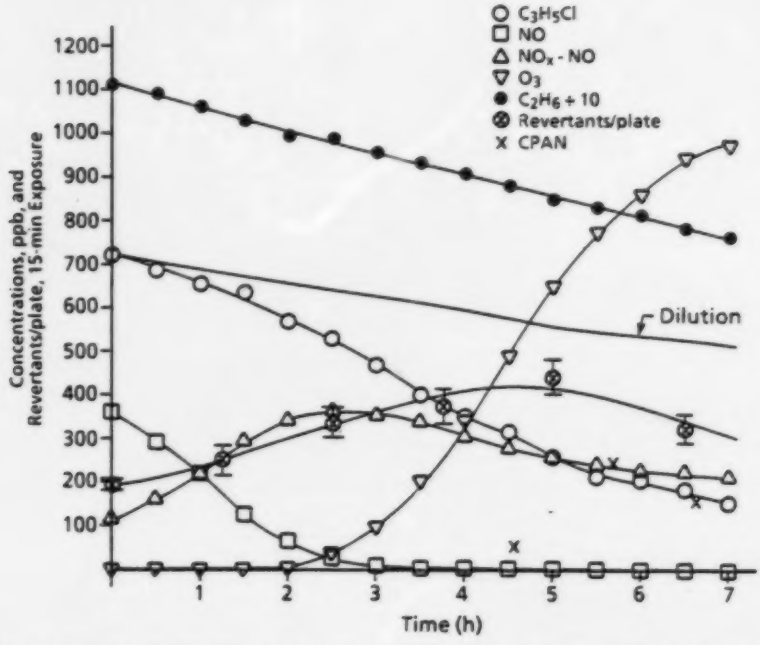

Figure 4.4-2. Experiment B: reaction chamber component concentrations and mixture mutagenic activity, $C_3H_5Cl/C_2H_6/NO_X$ irradiation.

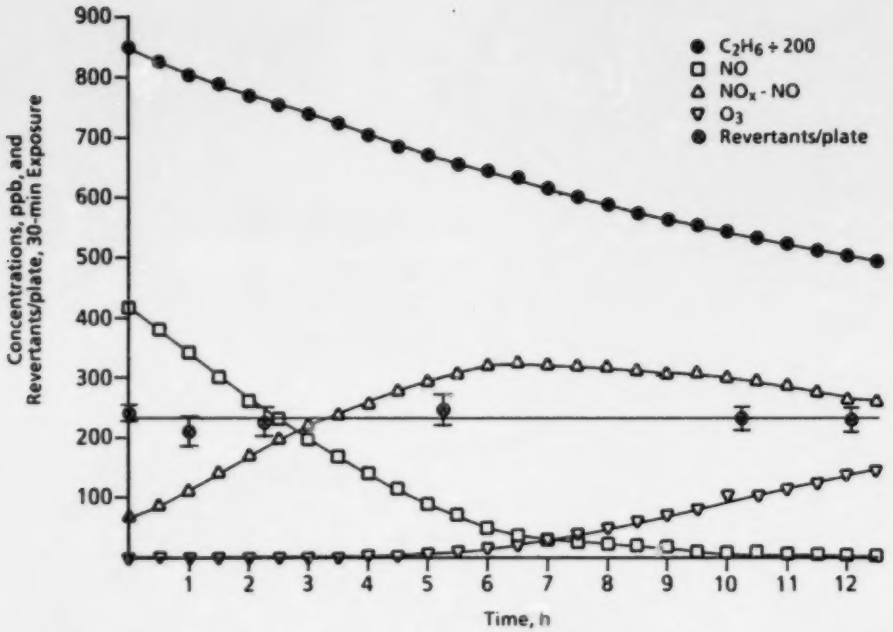

Figure 4.4-3. Experiment C: reaction chamber component concentrations and mixture mutagenic activity, C_2H_6/NO_X irradiation.

Since the dominant atmospheric removal process for allyl chloride is reaction with OH radicals, it would be desirable to measure the mutagenic activity of the products in the absence of any Cl-atom reaction. As described previously (32), a major product of Cl-atom reaction with allyl chloride is 1,3-dichloroacetone. In addition to the reactant and product concentration data presented in Figures 4.4-1–4.4-4, we made periodic measurements of the allyl chloride photooxidation products chloroacetaldehyde, formaldehyde, glyoxal, acrolein, and 1,3-dichloroacetone, and the ethane photooxidation products acetaldehyde, formaldehyde, and PAN. The measurement of these species was made

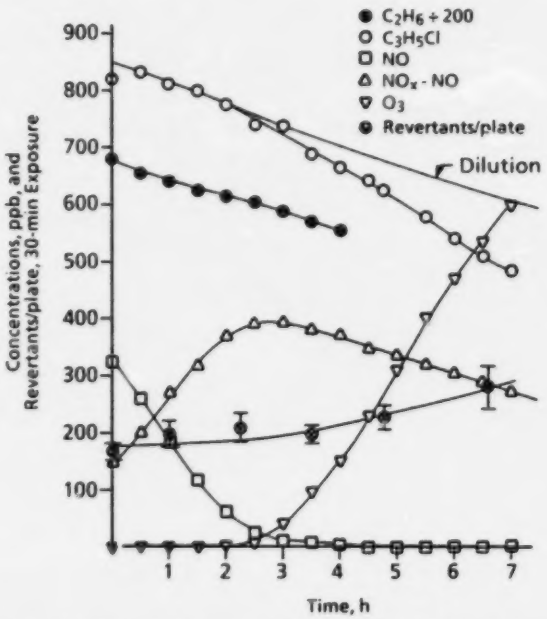

Figure 4.4-4. Experiment D: reaction chamber component concentrations and mixture mutagenic activity, $C_3H_5Cl/C_2H_6/NO_X$ irradiation.

roughly once each hour during each irradiation. To simplify the presentation, we provide in Table 4.4-1 the estimated maximum concentrations for these species during the reaction for each experiment. These estimates were obtained by plotting the actual data and drawing smooth curves through the product profiles. For Experiments A and B the product maxima occurred near the maximum in the Ames test results, and for Experiments C and D the maxima occurred at or near the end of the irradiation. Product profiles were presented for an experiment similar to Experiment A in a previous publication (32). From comparison of the 1,3-dichloroacetone data for Experiments A and B (and given the Δ allyl chloride data in Figures 4.4-1 and 4.4-2), it can be observed that the yield of Cl-atom addition products for Experiment B decreased by a factor of approximately six, which is in reasonably good agreement with our estimate based on the relative rate constants.

TABLE 4.4-1. REACTANT AND PRODUCT CONCENTRATIONS (ppb) AND BIOASSAY RESULTS

Experiment*	A	B	C	D
Reactants				
allyl chloride	729	720	–	820
NO	316	360	416	325
NO_x	445	472	483	474
ethane (ppm)	–	11.1	170	136
Products (max. conc.)				
O_3	925	978	148	600
formaldehyde	295	287	40	200
chloroacetaldehyde	132	58	–	117
acetaldehyde	–	101	104	196
1,3-dichloroacetone	75	7	–	≤0.5
acrolein	9	12	–	8
glyoxal	29	29	–	13
CPAN	80	190	–	no data
PAN	–	72	11	no data
Mutagenic Activity				
revertants·plate^{-1}·h^{-1}·ppb^{-1}	13	3.4	≤0.4†	1.4

* For Experiments A and B, the light intensity was 2.5 times greater than for Experiments C and D.
† Using Δ allyl chloride from Experiment D.

However, since measurable amounts of 1,3-dichloroacetone existed under these conditions, some of the observed mutagenic activity for Experiment B may still have been attributable to the Cl-atom chain products. The fact that the observed mutagenic activity decreased by a factor nearly equal to the decrease in the 1,3-dichloroacetone yield supports this idea. We have conducted standard plate incorporation tests with 1,3-dichloroacetone, and found it to be nonmutagenic for TA100. The observed mutagenic activity was, therefore, probably due to some other dichloro reaction product that resulted from Cl-atom addition to allyl chloride. A Cl-atom addition reaction is

assumed, since Cl-atom abstraction reactions would result in the same products as OH-radical abstraction reactions.

To be certain that all Cl atoms were scavenged by ethane we conducted Experiments C and D; in both cases the overall reaction was dominated by OH reaction with ethane, in contrast to Experiments A and B. (For Experiment D the initial OH reaction rate was 2.6 times greater with ethane than with allyl chloride.) For Experiments C and D the total plate exposure time for all exposure periods was 30 min. Under the initial conditions of Experiment D the rate of Cl-atom reaction with ethane is 45 times greater than with allyl chloride. Given that at the sixth exposure period for Experiment D $\Delta C_3H_5Cl = 145$ ppb, assuming that the Cl-atom yield from OH (and O_3) reaction with allyl chloride is 20% (32), and given the relative Cl-atom reaction rates, we estimate the total Cl-atom reaction with allyl chloride would correspond to a product concentration of 0.6 ppb (total Cl-atom reaction product). This estimate is consistent with the fact that the 1,3-dichloroacetone concentrations for this experiment were all below the 0.5-ppb detection limit.

Since much of the product mixture present in Experiment D represents ethane photooxidation products (i.e., HCHO, CH_3CHO, PAN [55]), Experiment C was conducted as a control for measurement of their mutagenic activity. As can be seen in Figure 4.4-3, the revertant counts did not increase significantly throughout the experiment.

For the sixth exposure period in Experiment D, at which time 145 ppb of allyl chloride had been consumed, the observed mutagenic activity increased slightly, corresponding to 100 excess revertants/plate (Figure 4.4-4). The observed mutagenic activity of the allyl chloride product mixture corresponds to 1.4 revertants·plate^{-1}·h^{-1}·ppb^{-1} (h^{-1} refers to per hour exposure). We note that under the conditions of the sixth exposure in Experiment D, some O_3 reaction with allyl chloride had occurred (we estimate 40-50% of the total Δ allyl chloride). Under these conditions, therefore, the observed mutagenic activity was smaller than that observed in Experiment B (in terms of revertants/ppb allyl chloride consumed) by a factor of approximately three, as a result of the decreased extent of Cl-atom reaction with allyl chloride.

From the data presented in Figure 4.4-1 (Experiment A), it can be seen that the mutagenic activity of the product mixture (at long extent of reaction) was approximately 13 revertants·plate^{-1}·h^{-1} per ppb of allyl chloride consumed. This mixture, then, is roughly 300 times more mutagenic than are the photooxidation products (0.043 revertants·plate^{-1}·h^{-1}·ppb^{-1}) of its nonchlorinated analogue, propylene. The majority of the mutagenic activity of this mixture is due to Cl-atom addition products, however. The mutagenic activity of the product of allyl chloride photoxidation in the absence of Cl-atom reactions (Experiment D) is considerably less (~1.4 revertants·plate^{-1}·h^{-1} per ppb), but is still roughly 30-40 times greater than

that for the photooxidation products of propylene. It will be shown in the Discussion section that under the conditions of Experiment D, the observed response can be accounted for on the basis of the mutagenic activity of the allyl chloride photoxidation product, chloroacetaldehyde.

4.5 COMPLEX MIXTURE IRRADIATIONS

To compare the results of the experiments with simple hydrocarbons with those of a more realistic simulated urban hydrocarbon mixture, and to determine the distribution of mutagenic species between the gas and particulate phases, we conducted a series of irradiated wood smoke/NO_X and automobile exhaust/NO_X experiments. For these experiments the hydrocarbon and total NO_X concentrations in the chamber were brought to the desired initial levels (12-20 ppmC and ~0.7 ppm, respectively), at which point the chamber was sealed off and the lights were turned on. The reaction was then allowed to continue until the maximum ozone concentration was reached, at which time the lights were turned off. Exposures of TA100 and TA98 to the gas-phase pollutants were conducted for periods up to 10 h, and filter samples of the particulate phase were obtained for bioassay measurements.

A set of duplicate wood smoke/NO_X irradiations and exposures (Experiments E and F) was conducted in which the initial total hydrocarbon concentration was ~17 ppmC and the initial NO_X concentration was ~0.62 ppm. Roughly 0.13 ppm of the NO_X was combustion derived, and the rest was added to the chamber from a compressed gas cylinder. NO_X was added to better simulate the hydrocarbon to NO_X ratio found in populated areas (24) and to increase the extent of reaction, thereby facilitating analysis of the species concentrations and determination of the mutagenic activities. Several additional static runs were conducted, mainly for chemical analysis, for which particulate-phase biological assay data were also obtained.

The reactant and product concentrations measured before and after the wood smoke irradiations are presented in Table 4.5-1. Concentrations of chemical species measured from the particulate extract data are presented in Table 4.5-2. In Figure 4.5-1 we present the reactant and product profiles for a number of major species. As shown in the figure the ozone maximum was reached in just 2 h, indicating that the photooxidation proceeded rapidly. As indicated in Table 4.5-1 the major reactive hydrocarbons included alkenes (ethylene, propylene, and 1-butene), aromatics (benzene, toluene, and xylenes), and oxygen-atom heterocycles (furan, 2-furaldehyde, and 2-methylfuran). Aldehydes were a large component of the gas-phase emissions from the wood stove, the two main species being HCHO and CH_3CHO. The aldehydes exist as both reactants and products in this system. The oxidation of propylene and toluene leads to the production of HCHO and CH_3CHO, as was discussed earlier. The photooxidation of aromatics (toluene and xylenes) has been shown to yield glyoxal (CHO_2) and methylglyoxal [$CH_3C(O)CHO$], and biacetyl [$(CH_3CO)_2$] (55), all of which are also present as reactants.

TABLE 4.5-1. INDIVIDUAL GAS-PHASE INORGANIC AND HYDROCARBON CONCENTRATIONS,* ppb, FOR WOOD SMOKE/NO_X IRRADIATIONS

Species	E (March 7)		F (March 28)	
	Initial	Final	Initial	Final
Nitric oxide	454	0	461	0
NO_X	657	252	576	259
Ozone	0	467	0	696
Carbon monoxide, ppm	38.0	33.4	38.7	35.5
Methane	5,060	4,500	4,480	3,920
Ethane	146	135	–	–
Propane	9	9	–	–
Ethylene	537	313	847	439
Propylene	126	8	100	7
1-Butene	58	3	39	0
Isoprene	–†	–	7	0
Acetylene	177	155	–	–
Benzene	62	50	102	68
Toluene	62	15	24	10
m- + *p*-Xylene	40	19	12	3
o-Xylene	13	4	2	0
Styrene	22	0	7	0
m-Methylstyrene‡	9	0	3	0
Furan	71	0	59	0
2-Methylfuran	61	0	64	0
Formaldehyde	269	365**	229	383
Acetaldehyde	88	109**	57	75
Benzaldehyde	15	12	–	–
2-Furaldehyde	31	2**	22	6
Glyoxal	41	45**	26	24
Methylglyoxal	41	36**	13	16
Biacetyl	–	–	7	5
PAN	0	174	0	232
Chloromethane	18	18	–	–
HC††, ppmC	16.4	13.2	17.2	15.0

* Other gas-phase species identified: phenol, butadiene, methyl acetate, 2-methyl vinylacetylene, methyl vinyl ketone, methyl ethyl ketone, and benzofuran.
† Not measured.
‡ Tentatively identified based on mass spectrum.
** At 1.5-h irradiation time.
†† Corrected for dilution.

TABLE 4.5-2. PARTICULATE EXTRACT DATA FOR WOOD SMOKE/NO_X IRRADIATIONS, NANOGRAM OF PAH/MILLIGRAM OF PARTICULATE MASS

Species*	E (March 7)		F (March 28)	
	Initial	Final	Initial	Final
Fluoranthene	721	70	611	203
Pyrene	672	50	560	218
Anthracene + Phenanthrene	457	100	132	89
Acenaphthene	371	30	103	≤ 100
Fluorene	214	20	224	≤ 100
Chrysene + Triphenylene	–	–	100	≤ 100
CNC ($\times 10^9$ particles/m^3)	33	28	13	10
EAA (nL/m^3)	286	591	72	265

* Other particulate-phase species identified: 4-hydroxy, 3,5-dimethoxybenzaldehyde; 4-hydroxy-3-methylbenzoate; 4-hydroxy-5-methoxybenzoic acid; benzo(a)pyrene.

Physical and chemical characterizations of the particulate phase were conducted both before and following irradiation. The number distribution of the aerosols, although not shown, was similar to that observed by other researchers (11,15); that is, the vast majority of unreacted particulates were less than 1 μm in size with the maximum in the number distribution occurring at approximately 0.1 μm. The maximum in the number distributions shifted to ~0.2 μm after irradiation. The volume distribution of the particulate reactants and products as measured by the EAA for Experiment F is presented in Figure 4.5-2. Although the absolute number of particles following irradiation is smaller than

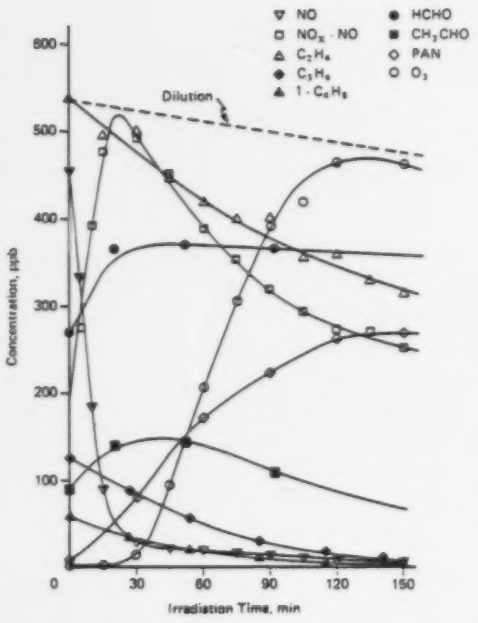

Figure 4.5-1. Wood smoke static irradiation with 500 ppb of additional NO_X. Selected product and reactant profiles.

before (Table 4.5-2), the contribution to the volume (and thus the mass) of the particulate matter increases as r^3, giving the volume distribution for products shown in Figure 4.5-2 with a peak at ~0.5 μm. This increase in volume following irradiation has been observed in all of our photooxidation experiments with wood smoke. It would seem likely that the increase in the total volume of particulate is the result of polar gas-phase photooxidation products adsorbing onto the particulate matter already present. In the absence of irradiation, the number and volume distribution maximum of the particles changed slowly with time while the particles were in the chamber. As shown in Table 4.5-2, for each of the PAHs measured in the particulate phase, significant degradation was observed as a result of the irradiation. Because the PAH concentrations are expressed as nanograms of chemical per milligram of particulate mass, part of the apparent degradation occurred solely as a result of the diluting effect of increasing the particulate mass (due to adsorption from the gas phase). However, there appeared to be some reaction of the PAHs, although no photolysis or reaction products from these compounds were observed.

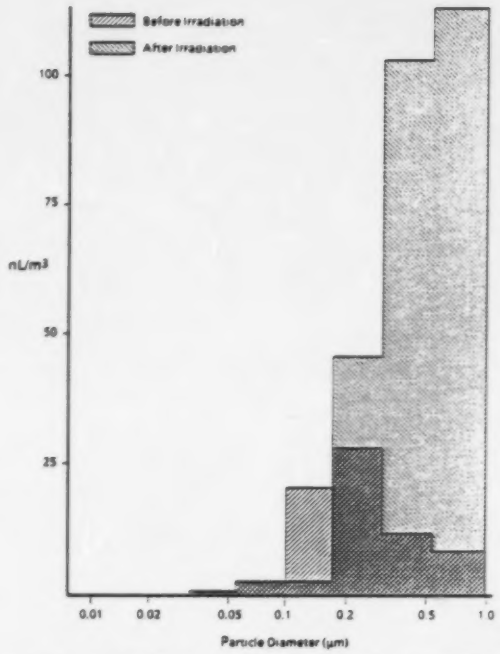

Figure 4.5-2. EAA volume distribution of diluted wood smoke before and following irradiation in Experiment F.

The mutagenic activities exhibited by Strains TA100 and TA98 from the exposure to the gas-phase species are presented in Table 4.5-3. The values represent the average of the slopes of dose-response curves obtained for Experiments E and F. Sample dose-response curves obtained with strains TA100 for Experiments E and F are presented in Figure 4.5-3. The exposure times indicated in this figure are corrected as indicated by Expression I (see Experimental section). In all cases, the clean air and ambient air revertant levels were (within experimental error) identical to the spontaneous laboratory controls (TA100:202; TA98:36). In Experiment E, data for an exposure time of 10 h (uncorrected) were also obtained. However, the irradiated mixture was toxic, as indicated by depressed revertant levels in the survivor plates. For all other exposure times, no other toxicity effects were observed.

TABLE 4.5-3. MEASURED MUTAGENIC ACTIVITIES† OF THE GAS AND PARTICULATE PHASES OF IRRADIATED WOOD SMOKE*

	Gas (revertants/h)		Particulate (revertants/µg)	
	TA100	TA98	TA100	TA98
Reactants	(1.6 ± 2.7)	(-0.4 ± 1.2)	0.30 ± 0.10	0.22 ± 0.06
Products	240 ± 22	45 ± 6	0.27 ± 0.15	0.94 ± 0.30

* Values in parentheses are zero within the uncertainty.
† Corrected for collection efficiency.

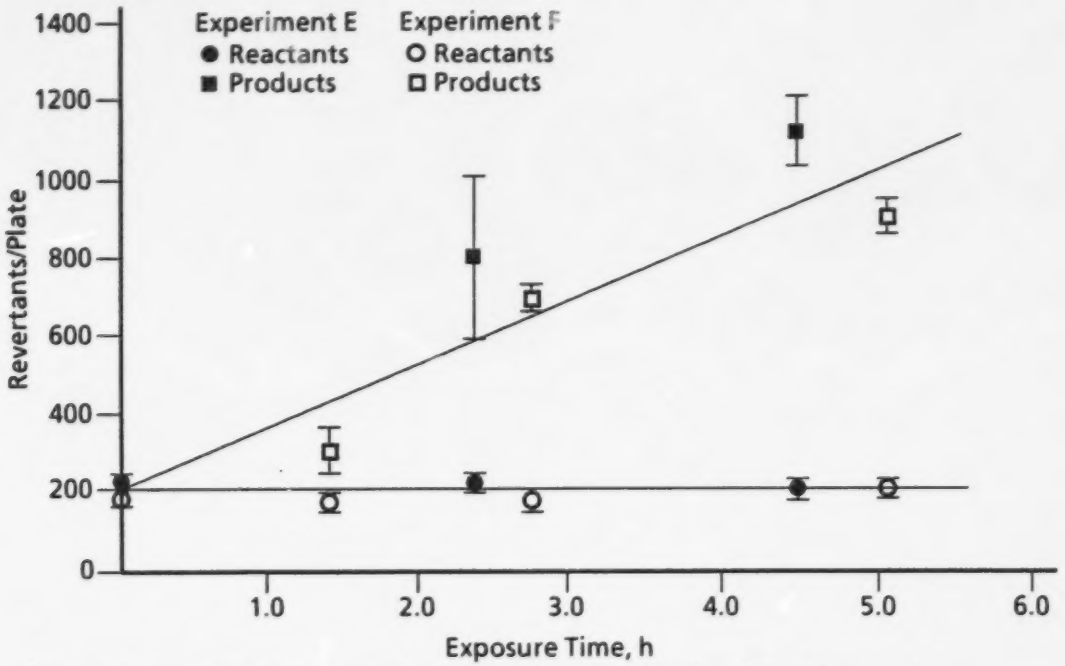

Figure 4.5-3. Dose-response curve for the mutagenic activity (TA100) of the gas-phase components of wood smoke (+ 500 ppb NO_X) before and following irradiation.

The mutagenic activities exhibited by strains TA100 and TA98 for the particulate organic phase are also presented in Table 4.5-3. For TA100 the extracts from the products show no increased activity over those of the reactants. Only for TA98 do the product extracts show significantly higher mutagenicity than the reactants (approximately a factor of 3), as shown in Figure 4.5-4. These particulate data are a compilation of filter extracts for all wood smoke/NO_X (added NO_X) irradiations performed during this study. The straight lines drawn through the data represent a least-squares fit to all the data.

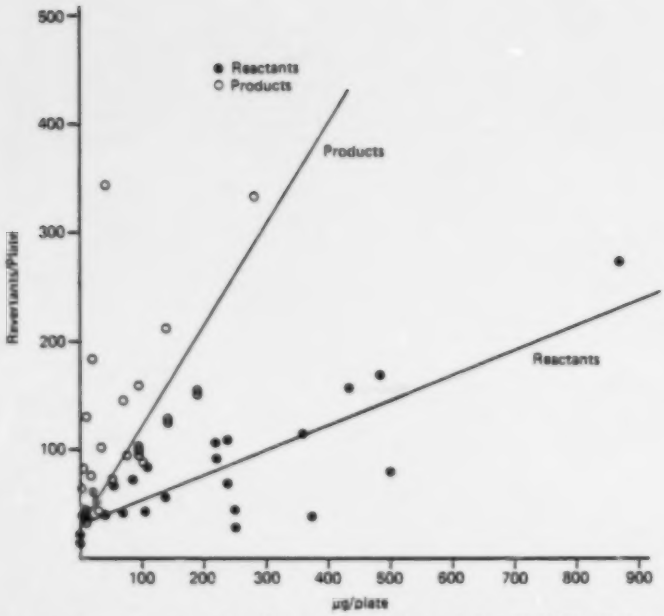

Figure 4.5-4. Dose-response curve for the mutagenic activity (TA98) of the particulate extracts of wood smoke (+ 500 ppb of NO_X) before and following irradiation.

We also performed exposures of the gas-phase reactants and products and plate incorporation tests for extracts using TA100 and TA98 with metabolic activation (S9). For exposures up to 10 h and extract doses up to 850 µg/plate, no significant difference was observed for the addition of S9 for either reactants or products in both strains when compared to the data without S9. In the interest of presenting results in the most succinct fashion, these data have not been presented.

It is apparent from the results of Experiments E and F that irradiation of wood smoke/NO_X mixtures dramatically increases the mutagenic activity over that of the initial reaction mixture, particularly for gas-phase species.

Another objective of this study was to attempt to estimate the fraction of mutagenic activity (revertants/microgram or revertants/cubic meter) present in the gas vs. particulate phase. The most convenient way to do this is to compare the mutagenicities on the basis of revertants per cubic meter. One major difficulty with this determination was obtaining an accurate measurement of the dose of gas-phase mutagens in the test plates. The number of revertants produced per cubic meter of effluent could be easily calculated, but we were not confident that all of the mutagens were removed by the bioassay chambers. We estimated the exposure chamber removal efficiency in an experiment that was a duplicate of Experiments E and F, in which the gas-phase exposures were conducted by using two exposure chambers in series. From comparison of the dose-response curves obtained from each exposure chamber, we calculated the collection efficiency of gas-phase mutagens for the first exposure chamber. From the slopes of the dose-response curves in this

experiment, the carryover into the second exposure chamber was found to represent 28 and 33% of the mutagenic activity observed in the first exposure chamber for strains TA100 and TA98, respectively. Assuming that the carryover from the second exposure chamber is represented by the same fractional values, the collection efficiency for the first exposure chamber is then 72 and 67% (TA100 and TA98, respectively). It should be noted that this assumes that all mutagenic species present deposit to some extent. The observed reversion rates for this experiment (corrected for collection efficiency) were 185 and 36 revertants/h for TA100 and TA98, respectively, in reasonably good agreement with those found for Experiments E and F. These collection efficiency values can now be used to accurately define the mutagenic activity (revertants/m^3) of the gas-phase product mixtures (see Discussion).

To understand potential reactions that may occur with wood smoke at night, we reacted 20.2 ppmC wood smoke (starved air conditions) with, nominally, 2 ppm N_2O_5, followed by a 7-h exposure of TA100 and TA98 to the resultant product mixture (Experiment G). The reactant and product concentrations, as well as the measured mutagenic activities of the gas- and particulate-phase products, are presented in Table 4.5-4. For this experiment, two product exposure chambers were operated in series, and therefore the gas-phase mutagenicity data take into account the observed collection efficiencies for each strain (63 and 55%, respectively, for TA100 and TA98). The extent of reaction can be related to the observed change in the total hydrocarbon concentration, 2.4 ppmC, which is somewhat less than that observed for the wood smoke/NO_X irradiations. The data in Table 4.5-4 show small increases in concentration for species expected to be products, such as HCHO and CH_3CHO, and some decreases for species reactive to NO_3, such as acrolein (56).

The most apparent and dramatic effect of the addition of N_2O_5 was an observed increase in particulate volume. In Figure 4.5-5 the EAA data for the wood smoke mixture before and after addition of N_2O_5 indicate that the total aerosol volume increased by a factor of 21, and the maximum in the particle size distribution increased from roughly 0.15 to 0.30 μm. This increase in particle size and concentration is similar to that observed for the irradiated wood smoke/NO_X mixtures, implying that significant amounts of gas-phase oxidation products (the equivalent of 1.7 ppmC) are adsorbed onto existing particulate matter. As indicated in Table 4.5-4 the gas-phase products exhibited mutagenic activities comparable to (and perhaps greater for TA100) those observed for the irradiated wood smoke/NO_X mixtures, even though the extent of conversion of reactants to products was somewhat less. In addition, the particulate-phase products were considerably more mutagenic, especially as measured with TA100, than the particulate matter produced from the irradiated mixtures. As observed with the irradiated mixtures, much of the decrease in PAH concentrations (ng/mg) can be related to the increase in the total particulate-phase mass. It is clear from this experiment that reactions of NO_3 and N_2O_5 with the gas- and particulate-phase components can lead to substantial increases in the mutagenic activity of the wood smoke mixture.

TABLE 4.5-4. WOOD SMOKE/N_2O_5 (EXPERIMENT G) REACTANT AND PRODUCT CONCENTRATIONS (ppb) AND PRODUCT MUTAGENIC ACTIVITIES

	Initial	Final (t_{rcn} = 20 min)
Gases		
Total hydrocarbons, ppmC	20.2	17.8
NO	18	0
NO_X	94	1,656
O_3	0	85
CO, ppm	19.6	19.6
PAN	5	16
C_2H_4	545	563
HCHO	343	394
CH_3CHO	158	182
Acrolein	173	123
Benzaldehyde	5	4
Glyoxal	24	25
Methylglyoxal	70	87
Particulate (ng/mg)		
Pyrene	161	≤ 20
Anthracene + phenanthrene	495	≤ 20
Fluoranthene	205	26
Total particulate, nL/m^3	20	420
Mutagenic Activities		
TA100, gases (rev/h)		148
TA98, gases (rev/h)		20
TA100 particulate (rev/μg)		1.2
TA98 particulate (rev/μg)		1.0

In our attempt to examine other complex sources found in populated areas, we also conducted an experiment for automobile exhaust (Experiment H) that was similar to the irradiated wood smoke/NO_X experiments described earlier. It was not possible to duplicate the initial hydrocarbon/NO_X ratios of the wood smoke experiments, however, because of the relatively high NO_X emissions in the automobile exhaust. Experiment H was conducted at a hydrocarbon/NO_X ratio of 16.5:1, which is closer to the typical urban HC/NO_X ratio of ~10:1 (24). The ratio of particulate matter to total hydrocarbon in the vehicle exhaust was 0.005 $nL \cdot m^{-3}$ $ppmC^{-1}$. Therefore, as indicated in Table 4.5-5, there was very little particulate matter in the chamber at the start of the irradiation. In Table 4.5-5 we present the gas- and particulate-phase reactant and product concentrations and measured mutagenic activities for Experiment H. The reactant mutagenic activity for the particulate phase was determined from a filter sample obtained at the tailpipe. As in previous experiments, two exposure chambers were operated in series, so the gas-phase mutagenicity data in Table 4.5-5 reflect the measured collection efficiencies (TA100: 74%; TA98: 50%).

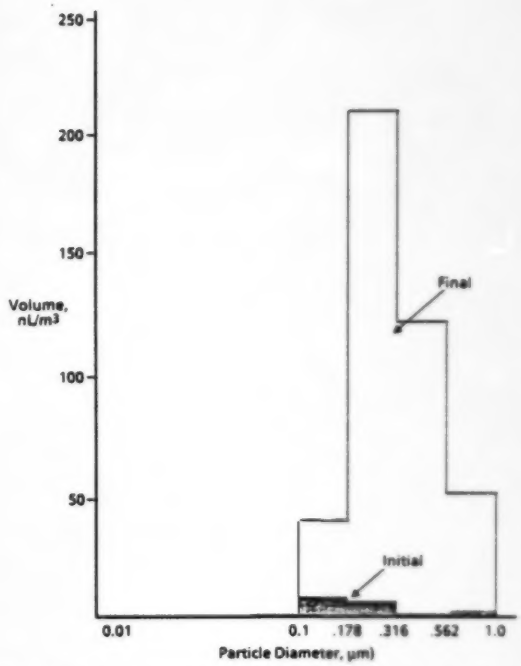

Figure 4.5-5. EAA volume distribution, wood smoke/N_2O_5.

The irradiation of the automobile exhaust/NO_X mixture was continued until the O_3 and PAN maxima were reached at 5.25 h. In contrast, the O_3 maximum for the wood smoke Experiment E was reached at 90 min, even though the HC/NO_X ratio was nearly identical to that for Experiment H. Therefore, it appears that the "reactivity" of the automobile exhaust mixture is somewhat less than that of wood smoke.

TABLE 4.5-5. IRRADIATED AUTOMOBILE EXHAUST/NO_X (EXPERIMENT H) REACTANT AND PRODUCT CONCENTRATIONS (ppb) AND MUTAGENIC ACTIVITIES

	Initial	Final
Gases		
Total hydrocarbon, ppmC	11.9	6.8
O_3	0	833
CO, ppm	19.6	15.2
PAN	0	149
NO_X	716	376
NO	668	0
Benzene	45	38
Toluene	71	52
C_2H_4	237	107
CH_4	580	470
C_3H_6	50	2
HCHO	30	213
CH_3CHO	25	79
Acrolein	0	62
Glyoxal	≤4	19
Methylglyoxal	≤6	55
Particulate (ng/mg)		
Pyrene	6,042	≤200
Fluoranthene	4,364	≤200
Anthracene + phenanthrene	11,571	≤200
Total particulate, nL/m^3	0.6	48
Mutagenic Activities		
TA100, gases (rev/h)	3	91
TA98, gases (rev/h)	≤2	18
TA100, particulate (rev/µg)	0.58	0.18
TA98, particulate (rev/µg)	1.0	0.40

In contrast with the wood smoke irradiation, in which the total number of particles decreased throughout the irradiation, a sharp burst of particle formation occurred in the automobile exhaust experiment shortly after the irradiation began (see Figure 4.5-6). Also shown are the NO, NO_X-NO, O_3, PAN, and total hydrocarbon concentrations measured as the reaction proceeded. The dramatic increase in particle concentration may be a reflection of the absence of particulate matter at the start of the reaction. That is, in the wood smoke irradiations the nonvolatile products condense onto existing particulate matter, while for the automotive emissions, new particles are formed.

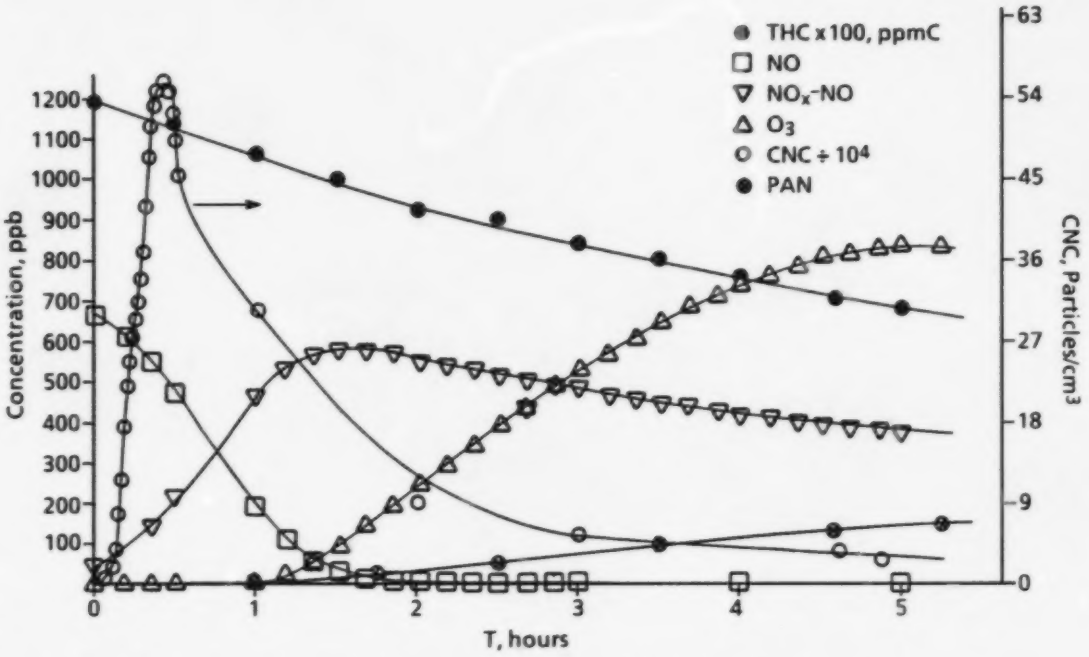

Figure 4.5-6. Automobile exhaust/NO_X reactant and product concentrations.

As indicated in Table 4.5-5, the mutagenic activities of the gas-phase products of irradiated automobile exhaust are similar to those measured for wood smoke. This seems fairly reasonable because, although the initial gas-phase concentrations are relatively lower for some species such as the aldehydes, the final concentrations of these species are similar. The particulate-phase mutagenic activities before and after irradiation are, however, significantly different from those observed for wood smoke. As shown in Table 4.5-5, the mutagenic activity (revertants/μg) of the automobile exhaust (reactant) particulate matter is considerably greater than that of the wood smoke particulate matter. In contrast to what has been consistently observed for wood smoke (measured by TA98), the mutagenic activities of the particulate-phase species decrease upon irradiation of the

mixture. Because essentially all of the particulate-phase mass represents condensed photooxidation products, these low-volatility products from the photooxidation of automobile exhaust appear to be less mutagenic than those produced from irradiated wood smoke mixtures. It is important to note that the total mass of particulate matter in the reactant and product automobile exhaust mixtures is much lower than for the wood smoke mixtures, and therefore the mutagenic activities of the automobile exhaust mixture on the basis of revertants per cubic meter will be correspondingly lower than those of wood smoke (see Discussion).

For our method, determination of the plate dosage presents a serious difficulty in quantifying gas-phase mutagenic activities. One alternative to our procedure is to collect the gas-phase components on a solid sorbent, followed by solvent extraction and utilization of the standard plate incorporation test (57). To compare the two methods, we conducted an additional wood smoke/NO_x irradiation in which the filtered effluent was drawn through a cartridge containing ~500 g XAD-2 and then into an exposure chamber loaded with the same number of TA98 and TA100 test plates used in the other experiments. In this way we could use the exposure chamber to measure any mutagenic activity that was not collected on the XAD-2. The experiment was essentially a duplicate of Experiment F described above. The initial total hydrocarbon and NO_x concentrations were 19.5 ppmC and 0.667 ppm, respectively, and the total reaction time was 65 min. The observed product concentrations were very similar to those indicated in Table 4.5-1 for Experiments E and F. After the irradiation, the exposure and the XAD-2 sample collection were conducted for 10 h at a flow rate of 14 L/min. By comparing the observed mutagenic activities from the exposure chamber plates with those expected according to similar wood smoke/NO_x irradiations, we found that XAD-2 removed 83 and 87% (TA100 and TA98, respectively) of the total mutagenic activity from the gas-phase airstream. Unfortunately, we estimate that roughly 60% of the gas-phase hydrocarbon removed from the airstream was lost in the collection and/or subsequent extraction and concentration procedures. The measured mutagenic activities (data not presented) of the XAD-2 extracts correspond to gas-phase mutagenic activities (revertants/m^3) that were roughly only 5 and 10% (TA100 and TA98, respectively) as large as the mutagenic activities measured with the direct gas-phase exposure technique. Therefore, although the XAD-2 collects or removes most of the mutagens, they appear to be lost in the sample workup procedures.

4.6 PEROXYACYL NITRATES

For the irradiation experiments with toluene, propylene, acetaldehyde, wood smoke, and automobile exhaust, PAN was a principal product near the ozone maximum where large mutagenic activities (TA100) were observed. It is important, therefore, to estimate the contribution of PAN to

the mutagenic activity of the complete system. We conducted a number of experiments by exposing TA100 to pure PAN in air, using the exposure chamber technique. Our first experiment involved preparation of PAN in the gas phase via irradiation of a $Cl_2/CH_3CHO/NO_2$ mixture, followed by purification of the PAN by vacuum distillation. Two separate exposures were conducted, using mixtures of 1 ppm (Exposure #1) and 3 ppm (Exposure #2) pure PAN in air. From the fraction of PAN that deposited into the plates, the total flow rate of the air mass, and the total exposure time, we calculated the total mass of PAN deposited. These numbers compared very well to those measured (as acetate and nitrite) in surrogate basic water plates. We used these values to construct a dose-response curve (58). The slope of this curve yielded a mutagenic activity for PAN of 34 ± 5 revertants/μmol. This value is such that, for example, if applied to the $\tau = 7.5$-h C_3H_6/NO_X experiments, PAN could account for no more than 10% of the total observed mutagenic activity of the product mixture. If this mutagenic activity were correct, we would be faced with a serious problem in interpreting the $\tau = 2.7$-h CH_3CHO/NO_X experiments, because PAN and HCHO are the only mutagens present and HCHO is a very weak mutagen (see Discussion). We therefore began to consider whether the observed mutagenic activity in these experiments could have been caused by synergistic effects involving the components present under the conditions of the short residence time CH_3CHO/NO_X experiment (i.e., CH_3CHO, O_3, NO_2, HCHO, CH_3ONO_2, and PAN).

To test for this possibility, we conducted a 10-h exposure of TA100 to a mixture of these products (at concentrations equivalent to those in the CH_3CHO/NO_X experiments) contained in the dark reaction chamber. The result of this experiment was that, for a 10-h exposure, there were roughly 200 excess revertants/plate, or a significant fraction of that observed for the irradiated CH_3CHO/NO_X mixtures. This experiment was then repeated in a series of experiments in which one of those species was removed in each successive experiment. In each case, a similarly large response was observed, as long as PAN was present. Our final experiment was a 10-h exposure of TA100 to ~200 ppb pure PAN in air. The results of this experiment are shown in Figure 4.6-1, where significant response can be seen (i.e., the equivalent of ~300 excess revertants/plate for a 20-h exposure). Therefore, according to this experiment, PAN could account for most of the observed mutagenic activity for the acetaldehyde and propylene experiments.

Given the quantity of PAN that was deposited into the plates in this experiment, we calculated a mutagenic activity for PAN of ~350 revertants/μmol (TA100), making it a moderately strong mutagen. However, as indicated above, in laboratory measurements using purified PAN we obtained a mutagenic activity of roughly a factor of 10 smaller. The only difference between this latter experiment and the first two pure PAN exposures (with regard to procedures) was the

considerably lower PAN concentration. From a series of exposures of TA100 to pure PAN at concentrations ranging from ~100 to 500 ppb, we observed that the observed reversion rate in revertants per hour is, within the experimental uncertainty, independent of the PAN exposure concentration. Specifically, for a number exposures in this range, the observed reversion rate was 14 ± 5 revertants/h. Therefore, the apparent mutagenic activity in either revertants per hour per ppb or in revertants per micromole calculated from any experiment depends on the exposure concentration. It is not, at this point, clear as to why this should be the case, because one would expect the calculated mutagenic activity to be independent of exposure concentration. For all these exposures, there was no evidence of serious toxicity problems (the dose-response curves were linear) or of Henry's Law equilibrium conditions being approached. However, in order to interpret the results from the irradiated HC/NO_X mixtures, the observed response can be compared with PAN's expected contribution of 14 revertants/h to the total reversion rate.

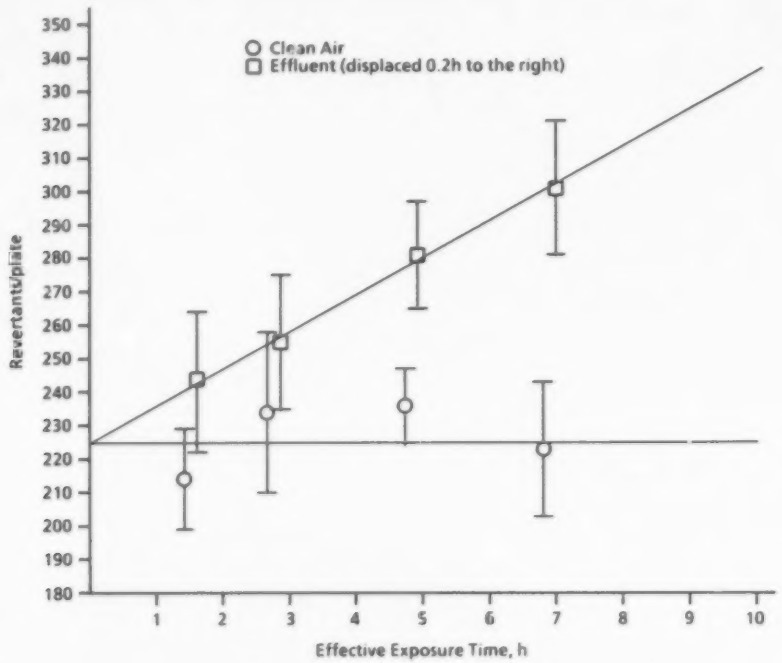

Figure 4.6-1. Dose-response curve for exposure of TA100 to pure PAN (220 ppb).

Measurements of the mutagenic activity of PPN, PBN, and PBzN have also been conducted. PPN and PBN were prepared according to the procedures of Gaffney *et al.* (36). PBzN was prepared in a dynamic-mode exposure involving a $C_6H_5CHO/O_3/NO_X$ mixture that produces PBzN and HNO_3 as the primary products. For these three PANs the mutagenic activities observed were 0.022, 0.016, and

0.097 revertants·h^{-1}·ppb^{-1}, respectively. It should be noted that these mutagenic activities are expressed per ppb of PAN compound in the inlet to the exposure chamber. For PAN, PPN, and PBN, the transfer efficiencies (to the exposure chamber) are all 65 to 70%, and the fraction that deposits is ~15%. For PBzN, the transfer efficiency to the exposure chamber was 27%, and the fraction depositing into the plates was 7%. Therefore, the actual dose for PBzN was ~5 times less than for PPN and PBN, indicating that the mutagenic activity for PBzN is considerably greater than for the other PANs. The inlet concentration for PPN, PBN, and PBzN was 500 ppb in each case. The apparent mutagenic activity of PAN at this concentration is ~0.028 revertants·h^{-1}·ppb^{-1}. Because it is unclear how the measured reversion rates for these other PAN-type compounds vary with the exposure concentration, it is not possible at this point to calculate a mutagenic activity for them in terms of revertants per micromole. It does appear, however, that the four peroxyacyl nitrates are mutagenic (as determined by using the Ames test, Strain TA100) to some extent. This is a significant result, given the fact they should all be produced under urban smog conditions. PAN and PPN have been routinely detected in urban atmospheres (59). These species are produced in the atmosphere primarily via OH reaction with the corresponding aldehyde (i.e., acetaldehyde, propionaldehyde, butyraldehyde, and benzaldehyde).

SECTION 5

DISCUSSION

In the Results section of this report it is shown that irradiation of the simple hydrocarbons acetaldehyde, propylene, and toluene in the presence of NO_X yields products that are significantly mutagenic, as determined by using the Ames test. It should be noted that none of these three hydrocarbon/NO_X mixtures are mutagenic prior to irradiation. This result suggests that common urban pollutants, which are themselves nonmutagenic, can be converted into significantly mutagenic products in the course of atmospheric photooxidation. All three of these species are important reactive components of urban air, so it is important to attempt to determine the cause of the observed mutagenic activities.

Throughout this discussion we will consider only the data for TA100 without S9. In all cases the reversion rate was higher with TA100 (and therefore easier to accurately measure), and no difference was seen between the data with and without S9 metabolic activation.

To determine which species may have caused the observed mutagenic activities for the irradiated mixtures it is necessary to know the dose of each product in the test plates and the mutagenic activity (in revertants·plate^{-1}·μmol^{-1}) of each product. The quantity of each species in the test plates can be determined from Expression II (see Section 4.2). To quantify the mutagenic activity of individual species, we conducted either standard plate incorporation tests with the pure chemical or single component gas-phase exposures in which the quantity of the species deposited into the plates was measured. Table 5-1 shows the mutagenic activities measured for all product species found to be mutagenic in this study.

For the irradiations of toluene and NO_X, the reaction products to be considered are PAN, NO_2, ozone, formaldehyde, acetaldehyde, methylglyoxal, glyoxal, benzaldehyde, the cresols, and nitric acid. The chemical mechanisms leading to these products have been described in detail elsewhere (28,47,60,61). The oxidation of toluene in this type of system occurs via an OH-radical chain mechanism. The OH radicals can either abstract an H atom from the substituent methyl group (e.g., to produce benzaldehyde) or add to the aromatic ring, leading to production of the cresols or a wide variety of ring fragmentation products including formaldehyde, glyoxal, and methylglyoxal. These ring fragmentation processes are quite complex, and a large degree of uncertainty exists regarding the predominant operative reaction pathways.

TABLE 5-1. MUTAGENIC ACTIVITIES MEASURED BY TA100

Species	Mutagenic Activity (revertants/μmol)
HCHO	12
Glyoxal	18
Methylglyoxal	200
H_2O_2	10
PAN	(14 revertants/h)*
2-HPN	3
2-NPA	3
$CH_3C(O)CH_2ONO_2$	55
CH_3OOH	(100)†
CH_2ClCHO	400

* Independent of concentration between 100 and 500 ppb. At 200 ppb this corresponds to ~680 revertants/μmol.
† Value found for $(CH_3)_3OOH$.

As suggested in the reaction profile (Figure 4.1-1) for the irradiation of toluene and NO_x, the product distributions for the two sets of dynamic experiments exhibit significant differences. At $\tau = 3.0$ h, NO in the system converts RO_2 radicals to RO radicals, thereby promoting the production of aldehydes and allowing for regeneration of OH radicals. At $\tau = 6.7$ h, the NO has been removed and NO_2 photolysis leads to buildup of O_3. Under these conditions, various radical-radical reactions can occur, leading to production of H_2O_2, organic peroxides, and peroxy nitrates. In addition, photolysis of the initially formed aldehydes will occur to a greater extent, and secondary reactions of the products with OH, O_3, and NO_3 can occur (e.g., cresols can be converted to nitrocresols).

These differences lead us to believe that at long extent of reaction the observed response for the toluene experiments may be caused by either peroxide-type compounds or aromatic nitro-compounds. We have found NO_2, O_3, acetaldehyde, benzaldehyde, the cresols, and nitric acid to be nonmutagenic. The test plate concentrations of HCHO, glyoxal, and methylglyoxal at $\tau = 6.7$ h were 0.93, 0.10, and 0.33 μmol/plate at the end of the 18.5-h exposure period (Table 4.1-2). Using the mutagenic activities of these three species listed in Table 5-1, we calculate that they contribute 11, 2, and 66 revertants/plate, respectively. Thus, methylglyoxal contributes roughly 13% of the total observed response at $\tau = 6.7$ h for TA100. Measurements were not made for H_2O_2. However, a reasonable upper limit concentration for this species would be 50 ppb, corresponding to ~0.64 μmol/plate if the entire mass deposited. Therefore, H_2O_2 contributes, at most,

6 revertants/plate, a negligible amount. As indicated in Section 4.6, exposures of TA100 to pure PAN in the concentration range of this experiment yields a reversion rate of 14 revertants/h. Dividing 500 excess revertants/plate by the total exposure time yields a reversion rate for the $\tau = 6.7$-h experiment of 27 revertants/h, assuming a linear dose-response curve. Therefore, PAN could account for up to ~50% of the total observed response. It should be noted that no survivor plates were used in the first toluene experiments, so it is unclear whether there may have been toxic effects for the $\tau = 6.7$-h experiments. The results of the subsequent 5 ppm toluene/0.9 ppm NO_X experiment indicate that this is certainly a possibility. That experiment yielded a considerably larger reversion rate (50 revertants/h), although it was conducted at shorter extent of reaction. In this latter case, PAN contributed roughly 28% of the total mutagenic activity, assuming 14 revertants/h for PAN's contribution to the reversion rate. Although we have accounted for a large part of the observed mutagenic activity for toluene, it appears that unidentified mutagens are present. It should be noted that several potentially mutagenic unsaturated dicarbonyl compounds (e.g., *cis*-butenedial, 6-oxo-2,4-heptadienal) have been found to be ring fragmentation products (46,47). Several terminal vinyl carbonyl compounds such as 1-pentene-3,4-dione and 5-oxo-1,3-hexadiene have also been found to be products of OH reaction with toluene (46,47). Vinyl carbonyl compounds can act as mutagens, presumably through an epoxidation mechanism occurring at the vinyl group (62,63). Finally, we note that in the absence of NO, hydroperoxides such as CH_3OOH are expected to be produced in small yields. As noted in the $(CH_3)_3OOH$ entry in Table 5-1, these hydroperoxides may be mutagenic.

In the irradiated C_3H_6/NO_X mixtures, the mutagens HCHO, 2-HPN, and 2-NPA are present at both residence times. However, calculations similar to those conducted for the toluene system indicate that these mutagens do not contribute significantly to the observed response. At $\tau = 7.5$ h , the measured reversion rates were ~24 revertants/h. Assuming a value of 14 revertants/h for the contribution from PAN, ~58% of the total observed mutagenic activity of the products of the photooxidation of propylene at long extent of reaction can be attributed to PAN. From the results of the C_3H_6/O_3 and C_3H_6/N_2O_5 experiments it is clear that ozonolysis products and the NO_3 reaction products do not contribute significantly to the observed mutagenic activity of the irradiated mixture. Thus, if other mutagens are present in this system, their identities are unknown. Again, it is possible that species such as CH_3OOH are present and are mutagenic. It should be noted that C_3H_6/NO_X gave a measurable response at $\tau = 2.7$ h. Although PAN is present at 6 ppb, we are currently uncertain as to whether it may have contributed to the observed mutagenic activity under those conditions.

It is interesting to note that products of the reaction of NO_3 with C_3H_6 were found to be mutagenic. It can be shown that $CH_3C(O)CH_2ONO_2$ is the only product measured that should contribute significantly to the observed mutagenic activity. Based on the calculated deposition for this species (0.4 µmol/plate), $CH_3C(O)CH_2ONO_2$ contributes 22 revertants/plate out of the total observed response of 180 excess revertants/plate, or ~12% of the total. It should be noted PAN is present at ~20 ppb. Unfortunately, we have not conducted exposures with pure PAN at levels this low, so it is unclear what its contribution might be. It is quite possible, however, that PAN contributes, to some extent, to the observed mutagenic activity of the C_3H_6/N_2O_5 mixture. It is known that organic hydroperoxides such as 2-HPPN (shown below) may be present and would be produced as shown in Reactions 25 and 26. These organic hydroperoxides may be significantly mutagenic, as is $(CH_3)_3OOH$.

$$C_3H_6 + NO_3 \xrightarrow{O_2} CH_3CH(OO)CH_2ONO_2 \qquad 25$$

$$CH_3CH(OO)CH_2ONO_2 + HO_2 \rightarrow CH_3CH(OOH)CH_2ONO_2 \qquad 26$$

In the case of the irradiated CH_3CHO/NO_X experiments at short extent of reaction ($\tau = 2.7$ h), the measured reversion rate was 12 revertants/h. We have repeatedly measured PAN's mutagenic activity as 14 (± 5) revertants/h for concentrations of 100-500 ppb. Assuming this value applies at the 102-ppb PAN concentration in this experiment, then PAN can account for all of the observed mutagenic activity for the $\tau = 2.7$-h irradiated CH_3CHO/NO_X mixture. This is the case for the long residence time experiment as well, within the experimental uncertainty. Of course, at the long residence time it is possible that other mutagens such as CH_3OOH are present as well.

Considering the magnitude of the observed mutagenic activity of the irradiated allyl chloride/NO_X mixture, it is certainly desirable to attempt to identify the mutagens that caused the response. From the data presented in Figure 4.4-1, it can be seen that the mutagenic activity of the product mixture (at long extent of reaction) was approximately 13 revertants·plate^{-1}·h^{-1} per ppb of allyl chloride consumed. This mixture, then, is roughly 300 times more mutagenic than are the photooxidation products (0.043 revertants·plate^{-1}·h^{-1}·ppb^{-1}) of its nonchlorinated analogue, propylene. If we assume that allyl chloride is converted to mutagenic products on a 1:1 molar basis, and that the total quantity of products deposit equally among the plates in the exposure chamber, it we can estimate the average mutagenic activity of the products in terms of revertants per micromole. Given eight plates per exposure, a 15-min exposure at 14 L/min, 1480 excess revertants at 4.0 h where $\Delta C_3H_5Cl = 440$ ppb, and making use of the ideal gas equation, we calculate a mutagenic

activity of ~3000 revertants/μmol for these products. The actual plate dose of products was probably smaller than that calculated, since some of the products (e.g., 1,3-dichloroacetone, CPAN) probably were not completely deposited, which would make the actual mutagenic activity of the mixture greater than that estimated above. In Experiment B the extent of Cl reaction with allyl chloride was decreased by a factor of 4-5 relative to that in Experiment A, and the mutagenic activity of the mixture decreased by a factor of 3-4. This suggests that the mutagen(s) that caused the majority of the response resulted from the reaction of Cl atoms with allyl chloride. Since the yield of Cl atoms from OH reaction with allyl chloride is ~0.2 (32), the dose of the Cl-atom reaction products in the plates is then ~5 times smaller than that used in the calculation above. The actual mutagenic activity of the products that caused the response is, therefore, at least 15,000 revertants/μmol. This represents a high mutagenic activity compared with other known strong mutagens (e.g., ~4000 revertants/μmol for β-propiolactone [9]). In addition, many of the products that do dissolve are not mutagenic, or are only very weakly mutagenic, such as formaldehyde and 1,3-dichloroacetone. This fact leads us to believe that the mutagenic activity of the product (or products) that caused the observed response is very large indeed. (We note that we are assuming in this discussion that the large response is not caused by synergistic effects.) The major product of Cl-atom reaction with allyl chloride, 1,3-dichloroacetone, is nonmutagenic, according to our standard plate incorporation tests. There are, however, other Cl-atom addition products that could be mutagenic and that are likely to be present, such as 1,3-dichloro-2-propyl nitrate, which is produced as shown in Reactions 27 and 28. It is reasonable to expect this product to be mutagenic, since 2-propyl nitrate is mutagenic according to our standard plate incorporation tests.

$$Cl + CH_2ClCH{=}CH_2 \xrightarrow{O_2} CH_2ClCH(OO)CH_2Cl \qquad 27$$

$$CH_2ClCH(OO)CH_2Cl + NO \rightarrow CH_2ClCH(ONO_2)CH_2Cl \qquad 28$$

Although it would be desirable to determine which species caused the observed response in Experiment A, the product distribution under these conditions is not representative of allyl chloride atmospheric photooxidation products. In the atmosphere the majority of Cl atoms are scavenged by other hydrocarbons. As indicated in Section 4.4, the mutagenic activity of the allyl chloride photooxidation products is nearly 10 times smaller in the absence of Cl-atom reactions (i.e., 1.4 revertants·plate^{-1}·h^{-1}·ppb^{-1} allyl chloride consumed). A calculation similar to that described above (for Experiment A) yields ~320 revertants/μmol for the product mixture (Experiment D) at the sixth exposure period.

Chloroacetaldehyde, one of the major products of OH reaction with allyl chloride, has been shown to be mutagenic (31). We conducted both standard plate incorporation tests and gas-phase exposures of TA100 to chloroacetaldehyde (in which the chloroacetaldehyde plate dose was quantitatively measured), and found values of 330 and 460 revertants/μmol, respectively. It would therefore appear that essentially all of the observed response for Experiment D can be accounted for by the presence of chloroacetaldehyde, whose concentration is roughly equal to $\Delta\ C_3H_5Cl$. (Interestingly, acetaldehyde is nonmutagenic when tested with TA100.)

It is likely, however, that there were also other mutagens in this mixture. For example, since PAN has been shown to be a mutagen when tested with TA100, it would seem reasonable to expect that CPAN is a mutagen as well. In addition, CPAN would be expected to be more soluble in the test medium than is PAN. However, under the conditions of these experiments, for a 15-min exposure and a 100-ppb PAN concentration, we would expect PAN to contribute no more than five excess revertants. Therefore, for CPAN to contribute significantly in these experiments it would have to be considerably more mutagenic than PAN. Preliminary experiments we have done with CPAN indicate that this is not the case. Other products we have identified, such as 3-chloroacrolein (32), may be mutagenic as well, although they have not yet been tested because of the unavailability of standards. Although glyoxal is a weak mutagen using this strain (~18 revertants/μmol), its concentration in this experiment is much too small to contribute significantly.

The *Salmonella* bioassay has been reported as being ineffective in the detection of chlorinated carcinogens (64). In a recent analysis of mutagenesis data, Claxton *et al.* (65) showed that the sensitivity with respect to detection of carcinogens using *Salmonella* for chlorinated organics is low (63%). However, specificity (measure of the fraction of noncarcinogens that yield a negative test result) has a quite high value of 91%. Therefore, a positive result with chlorinated organics is very meaningful since more than 90% of the *Salmonella*-positive compounds are animal carcinogens.

It is clear from these experiments that, although the photooxidation products of allyl chloride are much less mutagenic in the absence of Cl-atom reactions, they are much more mutagenic than are the photooxidation products of propylene (1.4 revertants $plate^{-1} \cdot h^{-1} \cdot ppb^{-1}$ vs. 0.043 revertants $\cdot plate^{-1} \cdot h^{-1} \cdot ppb^{-1}$, respectively). It may be that other, more abundant, chlorinated hydrocarbons yield more mutagenic photooxidation products than their nonchlorinated analogues. The fact that chloroacetaldehyde is a strong mutagen (as determined by using TA100) is in itself an important result because chloroacetaldehyde would be expected to be a common

photooxidation product of a variety of other chlorinated atmospheric hydrocarbons, such as chloroethene.

One objective of the wood smoke and automobile exhaust experiments was to determine the phase distribution of the mutagenic photooxidation products of irradiated complex HC/NO_X mixtures. From the results of the wood smoke/NO_X experiments in which the exposure chamber mutagen collection efficiencies were measured, it is possible to measure accurately the mutagenic activity of these mixtures for gas-phase species in terms of revertants/m^3, given the measured reversion rates (corrected for collection efficiency) in revertants per hour. This is calculated by using Expression III below, where R is the measured collection-efficiency corrected reversion rate in revertants per plate per hour, F is the total exposure chamber flow rate in cubic meters per hour, and N is the total number of exposed plates.

$$Mutagenic\ Activity,\ revertants/m^3 = R \cdot F^{-1} \cdot N^{-1} \qquad \text{(III)}$$

The mutagenic activity of the gas-phase products can also be estimated in units of revertants per microgram by dividing the mutagenic activity (revertants/m^3) by the gas-phase total hydrocarbon concentration in micrograms per cubic meter. This total hydrocarbon concentration can be calculated from the total hydrocarbon analyzer values in ppmC, using the ideal gas equation with the value 18.5 g/mol carbon for the average reaction product. The results of these calculations are presented in Table 5-2 for the irradiated wood smoke/NO_X mixtures (Experiments E and F). It is clear from the data in Table 5-2 that the mutagenic activity of the products of the wood smoke irradiation is much greater than for the reactants, except for the case of TA100 measurements of the particulate phase. In addition, gas-phase mutagens appear to be more important, relative to those in the particulate phase. On the basis of revertants per cubic meter, ~99 and 82% of the mutagenic activity was found in the gas phase (TA100 and TA98, respectively). The phase distribution of the mutagenicity is shown graphically in Figure 5-1.

TABLE 5-2. COMPARISON OF THE GAS- AND PARTICULATE-PHASE MUTAGENIC ACTIVITIES FOR WOOD SMOKE BEFORE AND AFTER IRRADIATION, STRAINS TA100 AND TA98

	TA100				TA98			
	Gas		Particulate		Gas		Particulate	
	Rev/m^3	Rev/µg	Rev/m^3	Rev/µg	Rev/m^3	Rev/µg	Rev/m^3	Rev/µg
Reactants	≤230	≤0.4	100	0.30	≤100	≤0.17	80	0.22
Products	17,300	1.6	180	0.27	3,230	0.30	730	0.94

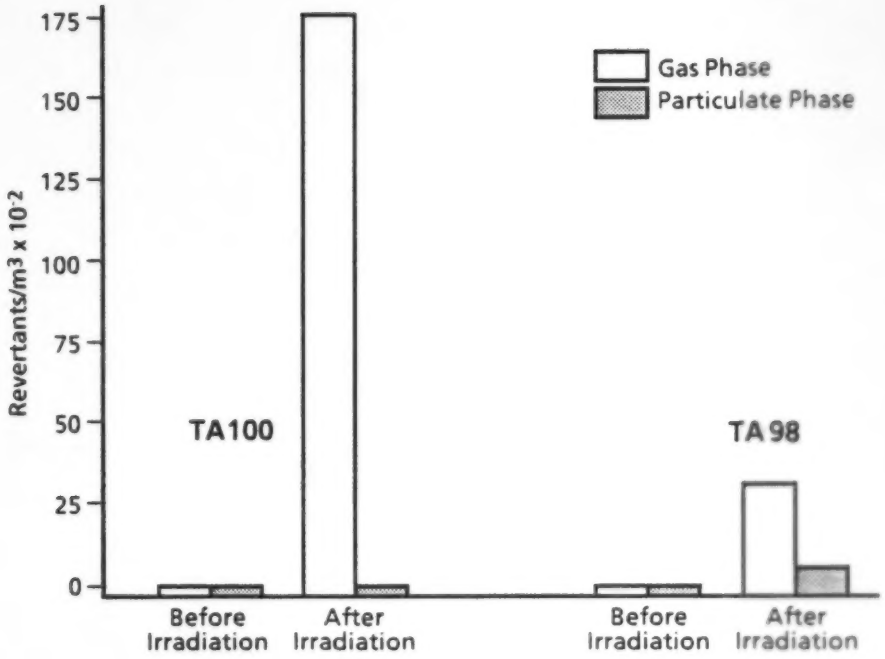

Figure 5-1. Comparison of gas- and particulate-phase mutagenicity of dilute wood smoke in air.

For the wood smoke/N_2O_5 experiment the mutagenic activities of the gas-phase products were 9510 and 1290 revertants/m^3 (TA100 and TA98, respectively). The corresponding numbers were 1620 and 1350 revertants/m^3 for the particulate-phase wood smoke/N_2O_5 products. The mutagenic activities of the products of the N_2O_5/NO_3 reaction with wood smoke are, then, comparable to or higher than (for particulate matter) those from the irradiated mixtures. This observation seems reasonable based on the current understanding of these reactions. For gas-phase species, NO_3 can react either by abstraction (e.g., for aldehydes to yield PANs) or addition to alkenes to form organic nitrates. The abstraction route produces the same products as the OH abstractions occurring in the irradiated system. We demonstrated earlier (Section 4.2) that NO_3 addition to alkenes such as propylene yields mutagenic organic nitrates. As discussed, reactions of N_2O_5 with particle-bound PAHs can yield mutagenic nitro-PAHs.

For the automobile exhaust/NO_X irradiation the mutagenic activities of the gas-phase products are 5850 and 1160 revertants/m^3 (TA100 and TA98, respectively). The mutagenic activities of the particulate-phase products are 28 and 62 revertants/m^3 (TA100 and TA98, respectively). These latter two numbers are clearly considerably smaller because of the much smaller total particulate mass present. On the basis of revertants per microgram, the particulate-phase products from the

simulation experiments with wood smoke and automobile exhaust mixtures are very similar (see Tables 4.5-3 and 4.5-5). On the basis of revertants per cubic meter, 99 and 95% of the mutagenic activity from irradiated automobile exhaust mixtures was found in the gas phase (TA100 and TA98, respectively).

These experiments indicate that irradiation of complex mixtures can produce substantial increases in the mutagenic activities for both gas- and particulate-phase products and that much of the observed mutagenic activity is present in gas-phase products. It would be desirable to determine the identity of the mutagenic products that are produced. Unfortunately, these mixtures are very complex prior to irradiation, containing a wide variety of reactive species, and are even more complex after irradiation, since each reactant can yield a number of different photooxidation products. Therefore, to identify each species that contributed to the observed mutagenic activities would be a formidable task. However, in the irradiated wood smoke/NO_x and automobile exhaust/NO_x experiments, PAN should contribute 14 revertants/h to the measured reversion rates of 174 and 70 revertants/h, respectively, measured by TA100. These are uncorrected reversion rates, because not all PAN deposits. PAN therefore accounts for ~8 and 20% of the total gas-phase mutagenic activity observed for these two systems (wood smoke, automobile exhaust, respectively). This is a reasonably large contribution for a single compound, given the complexity of the product mixture.

At this point, we have demonstrated that a number of atmospheric hydrocarbons can be converted to mutagenic products (as determined by using the Ames test) in the course of their photooxidation. An important question one might ask is, "What is the significance of this for ambient conditions?" To summarize the results of our studies with toluene, propylene, acetaldehyde, and allyl chloride, we can perform a simple calculation indicating the relative importance of these species in terms of the rate of production of mutagenic activity under atmospheric reaction conditions. This calculation is done with the aid of the typical ambient concentration data (24,59) and the rate constants (32,55) for their reaction with the OH radical that are presented in Table 5-3. The first row of this table presents the observed mutagenic activities for the products of the photooxidation of each of these species, in terms of revertants per hour of exposure, per ppb of hydrocarbon consumed, as measured in our simulation experiments. The relative significance of these mutagenic activities depends, therefore, on the rate of reaction of each of these species under atmospheric conditions. Values for the quantity (in ppb) of each hydrocarbon that would be consumed per hour of reaction for an OH concentration of 1×10^6 molecules/cm^3 (66,67) are given in the fourth row of Table 5-3. Finally, the number of revertants per hour of

exposure of TA100 to these products for these conditions can be obtained by multiplying the values in the first row of Table 5-3 by the values in the fourth row. These values are given in the last row of the table. It is important to understand that these values are the number of revertants per plate per hour of exposure, according to the exposure techniques employed in our experiments. The absolute magnitude of these values has little actual significance. However, the relative magnitudes represent their potential relative contribution to the production of atmospheric mutagens (i.e., bacterial mutagens). These values are listed in the table in order of decreasing importance. Allyl chloride is relatively insignificant because of its very low ambient concentrations.

TABLE 5-3. CALCULATED MUTAGENIC ACTIVITIES OF THE PHOTOOXIDATION PRODUCTS OF ATMOSPHERIC HYDROCARBONS

	CH_3CHO	Toluene	Propylene	C_3H_5Cl
Rev·h^{-1} ppb^{-1}	0.094	0.069	0.036	1.4
Urban conc., ppb	9	15	4	0.005
k(OH)·10^{11} cm^3 molecule^{-1}s^{-1}	1.62	0.62	2.63	1.7
k(OH)·[OH]·[HC] (ppb/h)	0.52	0.34	0.38	0.0003
Rev/h	0.049	0.023	0.014	0.0004

If the ~14-revertants/h reversion rate measured for 100 ppb PAN is applicable at ambient concentrations (typical urban mean concentrations are ~0.6 ppb [59]), then the mutagenic activity of PAN (according to our experimental conditions) is ~0.14 revertants·h^{-1}·ppb^{-1}. At ambient concentrations PAN then might contribute as much as 0.08 revertants/h, which is larger than the values calculated in Table 5-3. It must be noted, however, that the sensitivity of the Ames test according to our procedures is such that the mutagenic activity of PAN could not be measured at these low concentrations.

For each compound in Table 5-3, it should also be noted that the indicated mutagenic activities may not necessarily be contributed independently. The two largest potential contributors, CH_3CHO and PAN, are photooxidation products of toluene and propylene, and a portion of the mutagenic potential from CH_3CHO and PAN is already accounted for under the toluene and propylene columns. Clearly, however, other ubiquitous pollutants in urban atmospheres could give rise to these and other mutagens in the ambient atmosphere.

In conclusion, this work demonstrates that a variety of atmospheric hydrocarbons can be converted into mutagenic products in the course of their photooxidation. It is important to understand which species caused the observed mutagenic activities, particularly for reactive atmospheric hydrocarbons such as acetaldehyde. A few mutagenic products have been identified,

such as PAN, but much more work is necessary to determine the potential human health implications. We have shown in laboratory studies that irradiated HC/NO_x mixtures result in highly mutagenic products, and it would be worthwhile to attempt to measure ambient mutagenicities as a function of the photochemical pollutant concentrations. These results should be regarded as a first step in a long-term research effort. Much more work is clearly called for in assessing the extent to which hazardous compounds can be produced from the photooxidation of reactive hydrocarbons in urban air.

REFERENCES

1. U.S. Surgeon General. "Health Effects of Toxic Pollution: A Report from the Surgeon General"; 1980; Serial 96-15.
2. Haemisegger, E.; Jones, A.; Steigerwald, B.; Thomson, V. "The Air Toxics Problem in the United States: An Analysis of Cancer Risks for Selected Pollutants"; U.S. Environmental Protection Agency, 1985, EPA-450/1-85-001.
3. Haemisegger, E.R.; Jones, A.D.; Reinhardt, F.L. *J. Air Pollut. Control Assoc.* **1985**, *35*, 809-815.
4. Wise, S.A.; Chesler, S.N.; Hilpert, L.R.; May, W.E.; Rebbert, R.E.; Vogt, C.R.; Nishioka, M.G.; Austin, A.; Lewtas, J. *Environ. Int.* **1985**, *11*, 147-160.
5. Flessel, C.P.; Guirguis, G.N.; Cheng, J.C.; Chang, K.; Hahn, E.S.; Twiss, S.; Wesolowski, J.J. *Environ. Int.* **1985**, *11*, 293-301.
6. Schuetzle, D.; Jensen, T.E.; Ball, J.C. *Environ. Int.* **1985**, *11*, 169-181.
7. Schairer, L.A.; Van't Hoff, J.; Hayes, C.G.; Burton, R.M.; deSerres, F.J. In: "Application of Short-Term Bioassays in the Fractionation of Complex Environmental Mixtures"; Plenum Press: New York, 1979; pp. 421-440.
8. Bjeldanes, L.F.; Chew, H. *Mutat. Res.* **1979**, *67*, 367-371.
9. Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W.; Zeiger, E. *Environ. Mutat. Suppl.* **1983**, *1*, 3-142.
10. Wolff, G.T.; Countess, R.J.; Groblicki, P.J.; Ferman, M.A.; Cadle, S.H.; Muhlbarer, J.L. *Atmos. Environ.* **1981**, *15*, 2485-2502.
11. Dasch, J.M. *Environ. Sci. Technol.* **1982**, *16*, 639-645.
12. Ramdahl, T.; Alfheim, I.; Rustad, S.; Olsen, T. *Chemosphere* **1982**, *11*, 601-611.
13. Alfheim, I.; Ramdahl, T. *Environ. Mutagen.* **1984**, *6*, 121-130.
14. Pitts, J.N., Jr.; Lokensgard, D.M.; Ripley, P.S.; Van Cauwenberghe, K.A.; Van Vaeck, L.; Shaffer, S.D.; Thill, A.J.; Belser, W.L., Jr. *Science* **1980**, *210*, 1347-1349.
15. Kamens, R.M.; Rives, G.R.; Perry, J.M.; Bell, D.A.; Taylor, R.F., Jr.; Goodman, R.G.; Claxton, L.D. *Environ. Sci. Technol.* **1984**, *18*, 523-530.

16. Kamens, R.; Bell, D.; Dietrich, A. Perry, J.; Goodman, R.; Claxton, L.; Tejada, S. *Environ. Sci. Technol.* **1985**, *19*, 63-69.

17. Pitts, J.N., Jr.; Sweetman, J.A.; Zielinska, B.; Winer, A.M.; Atkinson, R. *Atmos. Environ.* **1985**, *19*, 1601-1608.

18. Pitts, J.N., Jr.; Zielinska, B.; Sweetman, J.A.; Atkinson, R.; Winer, A.M. *Atmos. Environ.* **1985**, *19*, 911-915.

19. Gibson, T.L.; Korsog, P.E.; Wolff, G.T. *Atmos. Environ.* **1986**, *20*, 1575-1578.

20. Bell, D.A.; Kamens, R.M. *Atmos. Environ.* **1986**, *20*, 317-321.

21. Kamens, R.M.; Fulcher, J.N.; Zhishi, G. *Atmos. Environ.* **1986**, *20*, 1579-1587.

22. Ames, B.N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347-364.

23. Claxton, L.D. *Environ. Int.* **1985**, *11*, 375-382.

24. Finlayson-Pitts, B.J.; Pitts, J.N., Jr. "Atmospheric Chemistry: Fundamentals and Experimental Techniques"; John Wiley and Sons, Inc: New York, NY, 1986.

25. Singh, H.B.; Salas, L.J.; Cantrell, B.K.; Redmond, R.M. *Atmos. Environ.* **1985**, *19*, 1911-1919.

26. Brodzinsky, R.; Singh, H.B. "Volatile Organic Chemicals in the Atmosphere: An Assessment of Available Data"; U.S. Environmental Protection Agency, 1983, EPA-600/3-83-027(a).

27. Carter, W.P.L.; Atkinson, R.; Winer, A.M.; Pitts, J.N., Jr. *Int. J. Chem. Kinet.* **1982**, *14*, 780-788.

28. Carter, W.P.L.; Lloyd, A.C.; Sprung, J.L.; Pitts, J.N., Jr. *Int. J. Chem. Kinet.* **1979**, *11*, 45-104.

29. Grosjean, D.; Swanson, R.D.; Ellis, C. *Sci. Total Environ.* **1983**, *29*, 65-85.

30. Cupitt, L.T. "Fate of HAPs in Air"; U.S. Environmental Protection Agency: 1980; EPA-600/3-80-084.

31. Rannug, U.; Göthe, R.; Wachtmeister, C.A. *Chem.-Biol. Interact.* **1976**, *12*, 251-263.

32. Edney, E.O.; Shepson, P.B.; Kleindienst, T.E.; Corse, E.W. *Int. J. Chem. Kinet.* **1986**, *18*, 597-608.

33. Pitts, J.N. Jr.; Atkinson, R.; Sweetman, J.A.; Zielinska, B. *Atmos. Environ.* **1985**, *19*, 701-705.

34. Gay, B.W.; Noonan, R.C.; Bufalini, J.J.; Hanst, P.L. *Environ. Sci. Technol.* **1976**, *10*, 82-85.

35. Nielsen, T.; Hansen, A.M.; Thomsen, E.L. *Atmos. Environ.* **1982**, *16*, 2447-2450.

36. Gaffney, J.S.; Fajer, R.; Senum, G.I. *Atmos. Environ.* **1984**, *18*, 215-218.

37. Claxton, L.D.; Toney, S.; Perry, E.; King, L. *Environ. Mutagen.* **1984**, *6*, 331-342.

38. Fed. Regist. **1979**, *44*, 233.

39. Cares, J.W. *Am. Ind. Hyg. Assoc. J.* **1968**, *29*, 405-410.

40. Stephens, E.R. *Atmos. Environ.* **1967**, *1*, 19-20.

41. Lonneman, W.A.; Bufalini, J.J.; Namie, G.R. *Environ. Sci. Technol.* **1982**, *16*, 655-660.

42. Johnson, J.R. "Organic Syntheses"; Wiley: New York, 1939; Collect. Vol. XIX, p. 64.

43. Kuntz, R.; Lonneman, W.A.; Namie, G.; Hull, L.A. *Anal. Lett.* **1980**, *13*, 1409-1415.

44. Shepson, P.B.; Edney, E.O.; Kleindienst, T.E.; Pittman, J.H.; Namie, G.R.; Cupitt, L.T. *Environ. Sci. Tech.* **1985**, *19*, 849-853.

45. Pitts, J.N., Jr.; Sanhueza, E.; Atkinson, R.; Carter, W.P.L.; Winer, A.M.; Harris, G.W.; Plum, C.W. *Int. J. Chem. Kinet.* **1984**, *16*, 919-939.

46. Dundei, B.E.; O'Brien, R.J. *Nature* **1984**, *311*, 248-250.

47. Shepson, P.B.; Edney, E.O.; Corse, E.W. *J. Phys. Chem.* **1984**, *88*, 4122-4126.

48. Bandow, H.; Okuda, M.; Akimoto, H. *J. Phys. Chem.* **1980**, *84*, 3604-3608.

49. Hendry, D.G.; Kenley, R.A. *J. Am. Chem. Soc.* **1977**, *99*, 3198-3199.

50. Hampson, R.F., Jr.; Gavin, D., Eds. NBS Tech. Note (U.S.) 1975, No. 866.

51. Japar, S.M.; Niki, H. *J. Phys. Chem.* **1975**, *79*, 1629-1632.

52. Atkinson, R.; Lloyd, A.C. *J. Phys. Chem. Ref. Data.* **1984**, *13*, 315-444.

53. McCoy, E.C.; Burrows, L.; Rosenkranz, H.S. *Mutat. Res.* **1978**, *57*, 11-15.

54. Atkinson, R.; Aschmann, S.M. *Int. J. Chem. Kinet.* **1985**, *17*, 33-41.

55. Atkinson, R. *Chem. Rev.* **1986**, *86*, 69-201.

56. Atkinson, R.; Aschmann, S.M.; Goodman, M.A. *Int. J. Chem. Kinet.* **1987**, in press.

57. Mumford, J.L.; Harris, D.B.; Williams, K.; Chuang, J.C.; Cooke, M. *Environ. Sci. Technol.* **1987**, 308-311.

58. Kleindienst, T.E.; Shepson, P.B.; Edney, E.O.; Claxton, L.D. *Mutation Res.* **1985**, *157*, 123-128.

59. Singh, H.B.; Salas, L.J.; Smith, A.; Stiles, R.; Shigeishi, H. "Atmospheric Measurements of Selected Hazardous Organic Chemicals"; U.S. Environmental Protection Agency: 1981; EPA-600/3-81-032.

60. Killus, J.P.; Whitten, G.Z. *Atmos. Environ.* **1982**, *16*, 1973-1988.

61. Leone, J.A.; Seinfeld, J.H. *Int. J. Chem. Kinet.* **1984**, *16*, 159-193.

62. Lutz, D.; Elder, E.; Neudecker, T.; Heushler, D. *Mutat. Res.* **1982**, *93*, 305-315.

63. Fishbein, L. *Proc. FDA Office Sci. Summer Symp., Struct. Correl. Carcinogen. Mutagen.*, 2nd, 1977, 8.

64. Hollstein, M.; McCann, J. *Mutat. Res.* **1979**, *65*, 133-226.

65. Claxton, L.D.; Stead, A.G.; Walsh, D. *Mutat. Res.* **1987**, in press.

66. Hewitt, C.N.; Harrison, R.M. *Atmos. Environ.* **1985**, *19*, 545-554.

67. Cupitt, L.T. "Atmospheric Persistence of Eight Air Toxics"; U.S. Environmental Protection Agency, 1987, EPA-600/3-87-004.